

Can ethics be taught?

ONE bishop and two canons of the Church of England; one 'philosopher and humanist'; the director of studies in arts at the Open University; three scientist/administrators, and three officers of the British Association for the Advancement of Science made up a study group on science and ethics whose deliberations see the light of day in *The Sensitive Scientist*, by David Morley, secretary to the group, just published by SCM Press at £1.95. Certainly study groups and working parties of such heterogeneity can be ways for individual members to broaden their horizons and make new friendships; but does the product in the form of an agreed report (with very social occasional notes of dissent) really help us to think more deeply and more clearly about ethical issues in science?

Can a report, for instance, take us along new roads such as has been done by individual writers in the past—Bronowski, Conant, Glass, Thorpe and Waddington, for instance? The answer must be, regrettably, no. There may have been individuals on the committee with strong views on particular matters, and every now and then their voices can be heard. But for the most part the group has had to content itself with relatively bland descriptions of the pros and cons of various courses of actions.

The report begins by considering some of the ethical problems in science at present—whether truth must out regardless of the consequences; the use of animals in research; selective treatment of spina bifida; food and population; expenditure on space research; specific and general issues in conservation; defence research. It then goes on to discuss possible educational improvements to make science seem to young people a less dogmatic and more human and social activity; factors involved in people's choice of research, and cost-benefit analysis.

The group makes no claim that the range of topics is comprehensive, but states that it simply reflects matters in which individual members had some expertise. Even so, there will be a few raised eyebrows at the lack of analysis of such contentious subjects as recombinant DNA (given only a passing mention) and the problems faced by scientists under repressive regimes. Admittedly both are rapidly moving issues and the pace of the committee seems to have been leisurely, but this can hardly

be regarded as an excuse for not even summarising what is involved. For the rest, the group too often come to the conclusion that there is a lot to be said on all sides of the argument and that decisions have to be made by individual people in individual circumstances guided as best as they can be by their own sense of what is right.

This is unexceptionable and a pleasing contrast to the dogmatism that pervades much of the social-responsibility lobby at present. But it all reads a little too much as if the group did not develop much of a dynamic of its own. Ideally one would have expected some powerful interdisciplinary points of view to have emerged as various members of the working party paired off to look at contentious issues in detail, but there is little evidence of this. Particularly disappointing is the low profile that seems to have been maintained by those who might be regarded as on the 'ethics' side of the working party.

One topic that was briefly raised deserves much wider attention—the question of the teaching of ethics within a scientific training. Scientists often talk about 'ethics' in the same way they talk about the 'scientific method'—as something they have absorbed by practice and experience rather than by education or reading. Maybe this is right; those who have been to courses on the philosophy and methodology of science are not noticeably more scrupulous scientists than those who just learnt by doing. But the merits of a short course in ethics would not necessarily be the production of a more ethical scientist exuding a glow of virtue in all his doings, but simply a scientist whose tentacles were more attuned to the broader implications of the work in which he was involved. For many scientists an ethics course, whilst interesting and a way of moving across disciplinary boundaries, would not have any obvious pay-off; but the same could be said for many student courses which are regarded as essential to the complete education of a scientist.

It would have been pleasing to recommend this book as possible foundation material for such a course, but it doesn't really have strong enough a point of view for the purpose. Ethics, it seems, is not a matter for a committee to deal with. □



Biomedical research: US looks to a mixed economy

David Dickson describes moves to put medical research planning on to a multi-year basis

THE SHOCK-WAVES of California's decision to slash its property taxes were felt in Washington last week when the House of Representatives made a 2% cut of \$800 million from the budget request of the Department of Health, Education and Welfare.

The House has left it to the administration to decide where the cuts—if they are not restored by action in the Senate—should fall. This has brought to a head debate within the department over how its increasingly-restricted budget should be distributed between its various responsibilities, including the support of biomedical research.

The department's role in supporting basic science had already been put under scrutiny by HEW Secretary Joseph Califano, following President Carter's budget statement which emphasised the need to redress the federal government's comparative neglect of basic research in recent years.

Largely in response to the executive branch's initiative—although also reflecting concerns expressed by scientists themselves that too much stress has recently been placed on short-term, targeted research—Dr Califano is now proposing to draw up a five-year programme for health services research.

Outlining his proposal to a recent meeting of the American Federation for Clinical Research in San Francisco, Mr Califano said that limitations on resources for basic research made it necessary to develop a better strategy of allocation. "A multi-year plan will serve to level out the annual appropriations fluctuations that make rational research planning so difficult", he said.

Mr Califano said there was a need for an "across the board" strategy for health research that assessed research and health needs in a systematic and comprehensive way. He hoped to publish a multi-year plan—probably for five years—in early 1980, preceded by wide discussion about the principles on which such a plan should be based.

Among the principles offered by Mr Califano himself were: the need to respect a scientist's freedom to pursue research topics not immediately relevant to social needs; adequate support for population-based life sciences such as epidemiology and biostatistics; ample research opportunities for young investigators; and the strong orientation of government-supported research towards improving the quality of health services.

The task of putting flesh on these

principles, and of establishing the framework of the national debate scheduled to take place over the next few months, has been given to Dr Donald Fredrickson, director of the National Institute of Health which was responsible for \$2.2 billion out of the \$2.6 billion spent by HEW in 1977 on research and development.

Dr Fredrickson is keen to maintain a coherent research spectrum, ranging from the basic biological and biomedical sciences at one end to the more "targeted" problems of health care at the other. "Don't try to Rothschild it all", he told an open meeting of his advisory committee at NIH last week, referring to the British government's attempts to transfer responsibility for applied medical research from the Medical Research Council to the Department of Health and Social Security.

However, in attempting to map out a strategy for biomedical research that fits into Mr Califano's overall objectives, Dr Fredrickson faces a major dilemma: how to combine the essentially quality control forms of guidance required by one type of research with the more direct forms of intervention and control appropriate to the other.

"My major concern is to find how close you can bring the scientific process to other missions of the department of a more regulatory or service nature. But our problem is keeping out of some of the entanglements involved in these latter areas, while meeting our social responsibilities as biomedical scientists", Dr Fredrickson said.

Many scientists welcome the stability that a multi-year strategy could give to research. Particular areas in which it is felt such a strategy could help are in the supply and maintenance of equipment, and in providing measures to ensure the continued recruitment of young scientists into a field where career opportunities are decreasing daily.

Some members of the advisory committee, however, were concerned at last week's meeting that, by concentrating on applied health research, NIH might be threatening the support of non-targeted areas, especially if there was no overall budget increase.

Others expressed a general wariness of attempts to apply a planning strategy to basic science. Dr George Palade, chairman of the cell biology section of Yale University Medical School, echoed a common sentiment when he said it was impossible to predict the



Fredrickson: "Don't try to Rothschild it all"

fields from which critical contributions to the solution of biomedical problems were to come and so, it was impossible to plan research in a rational way.

Yet despite growing acceptance in political circles that the "disease of the month" approach to medical research so popular in the early 1970s has succeeded only in frustrating public expectations, Congress is far from accepting that its best strategy should be to give scientists the money and let them choose how to spend it.

Politicians and scientists, for example, now frequently engage in mutual recrimination for the apparent failure of the "war against cancer" to come up with promised results. Perhaps more seriously, there is also growing concern about the back up which scientists do, or do not, provide to regulatory agencies such as the Environmental Protection Agency and the Food and Drug Administration.

On the one hand, academic scientists tend to claim it is unrealistic for such agencies to approach them with specific requests, such as better short-term carcinogen tests, before a full understanding has been achieved of the biological processes involved. On the other hand, the agencies themselves, faced with the need to make decisions based on inadequate data, complain that they are often cold-shouldered by the scientific community, and that their own scientific efforts suffer as a result.

Such are the issues with which Dr Fredrickson now has to do battle. Several of those at last week's meeting suggested that an appropriate strategy to present to Mr Califano was one that

would guarantee a certain level of support for the "science base" of NIH's activities, leaving research issues more related to health care to be discussed within the broader framework of HEW

priorities.

Dr Fredrickson has indicated that he personally favours such a "mixed economy" approach to research planning. "I do not believe in the rational plan-

ning of science beyond a very limited range", he told the meeting. But just how limited that range is—or should be—is likely to be the subject of heated debate in the months ahead. □

Canada announces major research boost

THE CANADIAN Government has announced a package of measures designed to stimulate research and development activities, the low level of support for which has recently been the target of widespread public criticism. The Government's declared intention is to raise the proportion of the nation's gross national product spent on R & D from 0.9% to 1.4% over the next five years.

Included in the package is an increase of \$5.35 million in the budget of the National Research Council to enable it to expand its programmes and establish university-based research centres. This should improve co-operation between Government, research and industry.

The plan also allows for an immediate increase of \$10m in the budgets of the three main grant-giving bodies over and above the budgets already announced for 1978-79. As a result, the budget of the newly-formed Natural Sciences and Engineering Council will increase by 12.4% to \$109.8m (rather than increasing by only 7.1%, as originally planned). Similarly the budget of the Medical Research Council will increase by 11.1% (rather than 5.8%), to \$63m, and that of the Social Science and Humanities Research Council will increase by 15% (rather than 7.8%) to \$33.1m.

In April, the Medical Research Council announced that, with only a 5.8% increase in funds budgeted for next year, it was having to terminate a fifth of its established research projects, and would only be able to fund 30%

of the grant applications which it had received and had judged worthy of support. The new money, whose distribution will be at the discretion of the council, means that such drastic cuts are no longer necessary.

Following a meeting of the council at the end of last week, a spokesman said that the increase would allow the council to continue to fund projects of national importance without having to cut into its research base.

In slight contrast to the MRC, both of the other research councils have been told that the extra money should be spent on research projects which are seen to be of national importance. This reflects the main goal of the Government's package, which is to increase the technological strength of Canadian industry.

Thus, in addition to the extra money given to the grant-issuing bodies, Mr Buchanan also announced that the Government would increase its contracting-out research programme in each of the next two years, and would spend \$6.8m to establish research centres to assist in the development of industrial research activities.

Furthermore the Government is planning to introduce a \$3m works programme to create jobs for scientists and technicians to undertake research projects at the request of industrial firms.

The long-term need for a major basic research effort to support a national technological development programme was also emphasised last week by Dr Josef Kates, chairman of the Science Council of Canada. In a

statement published with the council's annual report, Dr Kates says that Canada's previous emphasis on technology transfer has not allowed it to participate effectively in R & D efforts leading to successful innovations and developments.

Stressing the Science Council's demand for a policy of "technological sovereignty" as a strategy for achieving scientific, industrial and economic strength, Dr Kates said that part of this policy should include an ability to barter competently and toughly for technologies which could be used to enhance Canada's industrial base.

"Unfortunately, many of the scientific agreements negotiated between Canada and other countries seem to reflect a sense of diplomatic exchange rather than of genuine technology transfer", Dr Kates says.

Other members of the scientific community, however, while welcoming the Government's decision to increase funds for R & D, are concerned at the apparent emphasis on targeted research. They feel that, in the long-term, support for basic research is necessary to guarantee the success of targeted research.

"We are worried that the result of this emphasis may be to leave many university scientists out in the cold. Furthermore, if industry fails to make good use of the incentives it is being offered by the Government, this could ultimately damage support for research in other sectors", Dr John Kucharczyk, of the Canadian Federation of Biological Sciences, said last week.

David Dickson

Computer secrecy ban lifted

FOLLOWING widespread criticism of apparent attempts to infringe academic freedom, the US Department of Commerce announced last week that it had decided to lift a secrecy ban imposed on 21 April on a research scientist at the University of Wisconsin-Milwaukee who has been working on the security of computer-based data systems.

According to a Department spokesman, the ban—which was imposed when the research scientist, Dr George Davida, had applied for a patent for techniques arising from his unclassified research—had originally been re-

quested by the Department of Defense. It was withdrawn following an inquiry by Commerce Department officials. □

Fall in graduate enrolment

THE NUMBER of graduate students enrolled in science programmes in US educational institutions fell by 1% in 1977, following continual growth since 1973, according to figures released last week by the National Science Foundation.

A 2% increase in part-time enrolment to 113,700 was more than offset

by a 2% decrease in full-time numbers to 228,600, leading to a total decrease of 3,400 between the fall of 1976 and the fall of 1977—from 345,700 and 342,300.

Improved job opportunities for those without a PhD was one of the main reasons for the decrease, according to the NSF.

Other possible causes included a 35% reduction in the enrolment of war veterans because GI Bill benefits have expired, increasing tuition and living costs, and declining federal support to students in graduate science and engineering programmes. □

What caused the Seveso explosion?

SCIENTISTS investigating the disastrous accident which occurred at the Icmesa chemical plant in the Italian town of Séveso, on 10 July 1976 have so far failed to identify fully the cause of the explosion. This is the disturbing state of affairs at the end of two years of intensive research by chemists employed by Givaudan, the Swiss owners of Icmesa.

The accident happened in a reactor used for the production of trichlorophenol. Six and a half hours after the reactor had finished its run, an unexpected rise in temperature in the vessel resulted in a pressure increase sufficient to blow a safety valve on a vent pipe. In the ensuing discharge from the pipe was an extremely toxic byproduct of the reaction—2,3,7,8-tetrachlorodibenzo-p-dioxin (dioxin)—and this was deposited over Séveso and its environs.

The scientist leading the investigation for Givaudan is the company's chief chemist, Dr J. Sambeth. He told *Nature* of the difficulties he had encountered in his attempts to identify the sequence of events which led up to the reactor's discharging of its contents. His frustration is all too apparent and, needless to say, understandable. It is Sambeth who has to help with the preparation of Givaudan's defence for the forthcoming trial in an Italian court, which will deal with the accident. In a hearing which is likely to become a legal test case, Sambeth intends to argue that the accident at Icmesa was neither foreseeable, nor the result of negligence.

It is the view of a large section of the chemical industry that Séveso should never have happened. The political damage which has been experienced by Givaudan and its Swiss parent company, F. Hoffmann La Roche, is not restricted to them alone. Many giants of the chemical industry feel that they, too, have suffered as a result. Far greater demands have been made on them recently to curb pollution in general.

Givaudan are sensitive to the suggestion made in Italy that the company operated in Séveso because of the Italian pollution laws, which are less vigorous than those in Switzerland. They are quick to deny the accusation. Their plant at Icmesa, they say, had to work within the Italian law. What of the charge that they skimped on investment in the plant? Sambeth claims that the reverse is the truth, claiming that not only has Givaudan poured millions of Swiss francs into the site, as part of a programme of modernisation, but that figures are

available to prove it.

Against this background, it is hardly surprising that Givaudan are anxious to establish the causes of the accident. Sambeth reports that the company's scientists have concentrated their efforts on an attempt to reproduce the conditions in the trichlorophenol reactor on 10 July; as well as on a study of the operating procedure in use at Icmesa.

One of the questions which has been asked concerned the previous recorded accidents in reactors manufacturing trichlorophenol. Was Givaudan aware of them? Indeed they were, retorts Sambeth; claiming that it was this experience which governed both the design and operation of the Séveso plant. The reaction employed in the trichlorophenol reactor is the hydrolysis of tetrachlorobenzene to sodium trichlorophenolate using sodium hydroxide. Dioxin is produced as an unwanted byproduct during the reaction. There is a choice between two solvents for the hydrolytic process—methanol or ethylene glycol. Givaudan chose the latter, for methanol would have had a pressure of 20 Atmospheres in the reactor, and any pressure vessel forms a hazard to workers. Although yields are better with methanol, the company decided against it in view of its implication in two accidents which occurred in Germany in 1953.

But ethylene glycol also has its hazards. In 1968, it was the solvent used in a trichlorophenol reactor at the site of Coalite and Chemical Products of Bolsover, which exploded, killing one engineer and contaminating a further seventy-nine employees with dioxin. The heating system at Coalite used oil in a primary circuit, which was often at a temperature close to 300 °C. The optimum temperature for the reaction to produce trichlorophenolate is about 180 °C. Above this temperature, the danger of an exothermic reaction starting is very real, particularly when the solvent has been removed.

The critical temperature is 230 °C; and once the exothermic process has started, the temperature rises rapidly to about 410 °C. To avoid the danger of localised heating in their system, Givaudan used superheated steam, which they insist would never rise above 200 °C. In addition, they could 'flush' cold water through these heating coils in a matter of minutes to reduce any unwanted temperature rise. To cope with the possible problem of blockages in the vessel pipes, Givaudan allowed xylene to circulate through the system. The vent pipe which discharged the reactor contents in 1976



was set to do so at a pressure of 3.8 Atmospheres—the vapour pressure of xylene at 180 °C. According to Sambeth, this was an added safety factor, but one designed to cope with problems at the beginning of the reactor run and not at the end, which was when they occurred at Séveso.

Sambeth also states that the volume of the reactor solvent was also carefully controlled for safety reasons. Solvent is required to reduce the viscosity of the reaction mix, and it has been shown that conditions in the reactor become dangerous if the heat is retained after the solvent is removed. In view of this, Givaudan insist that only 50% of the solvent was removed after each run; and on the day in question, it was just 15%. The final recorded temperature on 10 July was 158 °C.

Sambeth reports that chemists at Givaudan have attempted to simulate the reaction conditions with 15% of the solvent removed. However, in spite of their heating the mix to temperatures between 160 °C and 217 °C for up to eight hours, they have been unable to start an exothermic reaction. Raising the temperature beyond this range, the chemists found a radical alteration in the properties of the mix. At 260 °C, the pH of the mix changes from alkaline to acid, and the solution becomes an insoluble suspension. These

changing conditions result in dechlorination of the trichlorophenolate and an increasing concentration of dioxin.

The easiest and most reliable method of assessing the quantity of dioxin released during the Séveso accident would have been to examine the reactor contents in the wake of the explosion. This was never done. A court order prevented anyone from investigating the reactor contents for fourteen months; all estimates made by the Italian authorities had to be calculated from the concentration of the chemical on exposed surfaces at Séveso. The figure arrived at was 0.5 to 1.5 kg. But the court order could

have had more serious consequences. Sambeth believes that it is unlikely that the reactor contents would have changed a great deal in the intervening months between accident and investigation of the reactor; but he concedes that a change is not impossible, and that it could complicate his research programme.

Two further possibilities had also to be studied by Givaudan. The ethylene glycol solvent might have decomposed, generating large quantities of hydrogen; or air introduced into the vessel might have formed peroxides on the reaction surface. Neither of these changes was observed in the laboratory.

With most of the options ruled out, Sambeth is indeed puzzled as to the cause of the Séveso explosion. He says that other chemical companies have been approached for assistance, but none has managed as yet to provide a satisfactory explanation of the events on 10 July. It is Sambeth's belief, in view of his investigations and of the original design of the trichlorophenol reactor, that Givaudan would be correct in considering the accident to have been unforeseen, and that the company was not guilty of negligence. It remains to be seen whether the court agrees with this.

Alastair Hay

European Patent Office poses problems for microbiologists

MICROBIOLOGISTS considering patenting a new microbiological process, microbially-produced end product or even a genetically-engineered micro-organism, face a maze of patent regulations daunting enough to discourage all but the most confident. In particular, the requirements of the newest arrival on the international patents scene, the European Patent Office, with regard to 'microbiological' patents, are being strongly opposed by prospective patentees.

The European Patent Office in Munich has been set up under the European Patent Convention to enable patent rights in all European countries to be gained by a single application instead of by separate applications to the national offices. However, prospective patentees, mainly from the pharmaceutical and chemical industries, object that the present Rule 28 of the Convention dealing with microorganisms does not provide sufficient protection for the patentee while the patent is under examination.

Under Rule 28 the applicant must deposit cultures of the microorganisms specified in the patent application (if the microorganism is "not generally available to the public") when the patent is filed. The deposit must be with an approved culture collection to which the public have access. Most national patent laws require deposition of a culture on the grounds that the 'invention' has not been sufficiently described if a culture is not available.

The objection to Rule 28 arises from the conditions, or lack of them, governing the release of the culture while the patent is being examined. As soon as the patent is first published, some 18 months after filing, the culture is available on request to any legally-entitled person who undertakes to use it for experimental purposes only. In some countries, including the USA and

Japan, cultures are not released, or are only released under certain conditions, until the patent is granted. The prospect of a potentially valuable culture falling into the hands of competitors at such an early stage in the application and without any compensation for subsequent commercial misuse, has alarmed the chemical and pharmaceutical industries which have formed an informal association—MICROPAT—to lobby the EPO for changes in the regulations.

At a recent meeting of industrial microbiologists and patent experts, S. Crespi, Patents Controller of the National Research and Development Corporation, urged participants to keep up the pressure for change. The EPO is at present "receptive" to representations for change, said Crespi. Since there is no obligation to file patents with the EPO rather than the national patent offices the EPO undoubtedly wishes to avoid a boycott by its potential customers.

MICROPAT wants to limit the availability of cultures for which patents have not yet been granted. They have suggested that for residents of the countries specified in the patent application, the culture would be available at the time of first publication (18 months after filing the application) provided there is compensation for any commercial misuse of the culture before the patent is finally granted, which can take several years.

The UK national law does give such retrospective rights. In countries where such protection is not available, the culture should not be released until the second publication (when the patent is granted), as in Japan and the USA. MICROPAT also wants access to cultures by non-residents of the countries covered by the application to be restricted. Their only access should be through an independent resident inter-

mediary who would be able to carry out experiments and communicate the results to them.

The requirements of the EPO also pose problems for the culture collections themselves. For the purposes of the EPO an 'approved' collection is one with which the EPO has made a contract. As the regulations stand, an approved collection would then have no right to refuse a culture on the grounds that it was too dangerous or difficult to handle. This has led to no contracts yet being agreed between EPO and the British culture collections—notably the National Collection of Industrial Bacteria—who are at present negotiating with the EPO for such rights of refusal.

Who would bear legal responsibility in the case of accidental loss or damage to the cultures also worries the collections. This will have to be clarified before agreements are signed. Dr I. J. Bousfield drew a distinction between the case of the EPO and the requirements of the Budapest Convention, 1977 (not yet ratified). This provides for a single deposition in a recognised 'International Depositary Authority' to be sufficient for a patent to be filed anywhere in the world. In this case however the legal responsibility rests squarely on the governments of the countries concerned.

The requirement to release the culture to any legally-entitled person also raises ethical problems for the culture collections if the organism in question is a potential pathogen, and they are aware that the person requesting it is not competent to deal with it. The National Type Culture Collection, which deals with medically important bacteria, is reluctantly facing the prospect of dealing with 'patentable' microorganisms for the first time, since the Health and Safety at Work Commission require that a culture of any

potentially pathogenic organism which might be used industrially must be deposited with them.

The difficulty of proving identity between cultures, exaggerated by the propensity of microorganisms to change in culture, poses practical and philosophical problems for patent law. The ability to specify genetic sequences precisely in terms of their nucleotide sequence could provide a basis for criteria of identity, as Dr R. Hill of the National Type Culture Collection pointed out, but the problem of what constitutes a sufficient difference in patent law would still remain.

Patent law has not yet got to grips with the problem of genetically-engineered bacteria, both in terms of their patentability and of the particular

difficulties there might be in storing bacteria whose claim to originality lies in the properties possessed by a plasmid, which in some circumstances could be lost from its bacterial host. What should be patented—the recombinant plasmid itself or the combination of plasmid and bacterium? When will the techniques of genetic manipulation be sufficiently standard and the outcome sufficiently predictable for the product not to be patentable without some further original twist? G. S. A. Szabo, Patents Manager for the Wellcome Foundation, encouraged his audience to test the patent system with applications. As always however it looks as though the chief beneficiaries will be the lawyers.

Eleanor Lawrence

Sweden's sunny future

How might Sweden's energy be supplied in the year 2015? A report, 'Solar or uranium—to choose an energy future', shortly to be published by the Secretariat for Future Studies, describes two alternatives. Each costs the same and each gives everyone in the country the material standard of living of today's top 10% of the population. The uranium alternative would mean 70 nuclear reactors, half of them breeders. Under the solar alternative, energy would come from biomass, solar cells, hydropower, windpower and solar heating.

The assumptions in the report are that the population in 2015 will be the same as it is today—about eight million; that energy demand will be reduced to 80% of today's in industry (which agrees with the official prognosis for the year 1995), to 50% of today's in the service sector (transport of people and goods, commerce and government services such as education), and to 70% of today's for housing (mainly space heating).

Under the solar alternative, 351 TWh would be supplied by biomass and converted to the demand sector by fuel cells, the production of methanol and district heating cogeneration. The energy plantations needed would cover 2.9 million hectares (about 6–7% of Sweden's total land area), with an average production of 90 MWh per hectare per year. Solar cells would

supply a total of 50 TWh. Some would be connected directly to their loads; others would be used for the production of methanol; still others for seasonal storage of energy. Hydropower would yield 65 TWh, as compared to its present 57 TWh (a limited expansion, taking into account environmentalists' objections to the building of more plants). Windpower would contribute 30 TWh from 3700 units. Solar heating would be mainly used for space heating and would supply 71 TWh. The sun shines on Sweden for 1,500–2,000 hours a year (40% as much as North Africa), but because practically all of them are between May and September, storage systems would be needed to keep summer's heat for winter. The authors estimate that a 500,000-cubic metre reservoir of water would be able to store enough heat for several apartments in a district heating system.

At the moment, renewable sources provide 24% of Sweden's energy. Assuming that the first production units in all the renewable-source systems could be operating in 1990, the report foresees them providing 50% of the country's energy by the year 2000 and 100% by 2015.

The actual setting up of such a system would be far from simple, given the political pressures that exist for expanding nuclear power. Some of the possible snags the authors discuss are their assumptions that the research and development now being done into renewable energy technologies will be successful, and the inevitable clashes over land use. But it was partially to point out the snags of both energy systems that the authors, engineers Peter Steen and Måns Lönnroth and theoretical physicist Thomas Johansson, wrote the report.

Wendy Barnaby

Success for unified field theory

"I think the unification is there and we really have three forces of nature" said Abdus Salam last week. He was referring to a result, a closely guarded secret, announced last Monday (12 June) at the Stanford Linear Accelerator Center in California, which vindicated the unified field theory of the weak and electromagnetic interactions developed independently in 1967 and 1968 by himself and Steven Weinberg.

Three hundred people crammed into the SLAC seminar room to hear the result, announced by Dr Richard Taylor and his group. Even Salam (of Imperial College, London) and Weinberg (of Harvard) had not been told what the result would be. In the event, their theory was confirmed, there was not a single question on experimental technique, and the experimenters received a standing ovation.

Dr Taylor also lead the group which in the late 1960s had shown that there were tiny, hard objects within protons and neutrons. These are now universally believed to be quarks. In that experiment Taylor had fired high energy electrons at protons and measured their scattering, rather as Rutherford had fired alpha particles at atoms and discovered the nucleus. This time the electrons were polarised—with their intrinsic spin pointing in a fixed direction—but otherwise the experiment was the same.

The polarisation enabled the group to determine if the interaction of electron and proton showed any distinction between right and left. The Weinberg-Salam model predicted that it should, and in conjunction with neutrino experiments which had set the scale for the so-called 'parity breaking' between right and left, there was a precise prediction of the amount of parity breaking that should be seen in the SLAC experiments. Just this amount, which comes from interference within the model between the parity-violating weak force and electromagnetism, was observed.

"In a sense a chapter is closed" said Salam. Neutrino experiments had been converging on the Weinberg-Salam model. "The degree of agreement was uncanny". But two atomic physics experiments, measuring the same effect as the SLAC experiment, disagreed with the neutrino results, so all was not roses. These results still stand, but high energy physicists will feel more inclined to accept the rather clean experiment from SLAC, than the ingenious, but complex, experiments from Oxford and Seattle. More about this in *News and Views* soon.

Robert Walgate



Fellowship for wife of Romanian President



LAST week's state visit to Britain of President Nicolae Ceausescu, of Romania, and his wife was exceptional in its scientific implications—for Dr Elena Ceausescu is a scientist in her own right and, as Director-General of the Central Institute for Chemical Research in Bucharest, responsible for all research and development activities in chemistry in Romania and the implementation of new technology in the Romanian chemical industry.

The inauguration of Dr Ceausescu as a Fellow of the Royal Institute of Chemistry was not, therefore, simply a formal honour paid to a visiting VIP. (Had that been the case, she could have been created an Honorary Fellow). Dr Ceausescu applied for Fellowship in the regular manner, submitting her published research on macromolecular compounds, and the chemistry of polymers and rubber. Certainly, it would be idle to pretend that the ceremony of inauguration was arranged without reference to her husband's position—not all new Fellows are escorted to the Institute by a party of diplomatic personnel—nor do they merit a champagne reception afterwards. Within the framework of diplomatic procedure, however, Dr Ceausescu's speech was that of a scientist rather than of the wife of a head of state. Even her remarks on peace and disarmament were framed in a scientific rather than a political context—referring to the vast expenditure of “scientific potential . . . for the manufacture of means of mass destruction”, and the deep interest of scientists “that the fruit of their work serve the welfare and happiness of the peoples and not destruction and war”.

In his speech of welcome, the Presi-

dent of the Institute, Professor R. O. C. Norman noted that so far “our links with chemists in Romania have not been close”. Dr Ceausescu replied: “Personally I consider that the distinctions with which you have honoured me today oblige me to work for the strengthening of Romanian-British collaboration in the field of science in general and in that of chemical research in particular”.

In fact, a number of exchange agreements already do exist. There has been an Romania-British intergovernmental agreement in existence since 1967, and a long-standing cooperation agreement between the Romanian Academy of Sciences and the Royal Society. Several technology agreements have already been signed in connection with the visit, and a number more are expected in its aftermath.

What, however, are the prospects for joint research under such agreements? According to an official publication of the Romanian National Council for Science and Technology (a body charged in law with “ensuring fulfilment of party and state policy in science and technology” and directly subordinated to the Central Committee of the Romanian Communist Party and the Council of Ministers) Romanian science is highly target-orientated. R and D is organised on the basis of economic self-sufficiency and financed, in the case of short-term projects, by contracts with industry. In the case of research where the pay-off point must be estimated in terms of decades, and also inter-disciplinary research projects, the research may be supported from the state budget directly—but this, too, is on a contractual basis.

What then of basic research and

serendipity? Are they not, at least to some extent, disregarded by the planners? Not according to one scientific spokesperson, who stressed, however, that he was speaking entirely unofficially. He told *Nature* that basic research also had an important part to play, but that one must understand the underlying philosophy behind Romanian science policy. In the past, he said, Romanian science had been strong on the theoretical side, but there had been a failure in implementation. What was needed now was an expansion of applied science, to match the rapid expansion of Romanian industry.

Speaking of the various agreements signed in connection with the state visit, he noted that the Ministerial co-operation agreement on transport would cover quite a considerable amount of what might at first glance appear to be basic research, ranging from the mathematical modelling of traffic flows and optimisation problems (applied to containerisation) to environmental pollution and even sociology. A similar Ministerial agreement on agriculture, he said, would cover the stimulation of seed, storage of crops, and the logistics of fertiliser application.

Clearly, in any programme of mutual cooperation, both sides must make concessions to the “research philosophy” of the other. To British scientists, the Romanian statute that (according to Decree 689/1973 and Law No. 21/1974) scientific research staff including heads of sectors and laboratories are periodically “re-certified according to their positions and their contributions” in order to maintain “dynamic performance” must seem more than a little alarming. Doubtless such “recertification” periodically eliminates much academic dead wood, but what of the unfortunate scientist who fails to arrive at even a negative result in the stipulated time? The spokesperson explained that it is not simply research results which are taken into account, but personal publications, keenness in keeping up one's reading and awareness of current trends, and general demeanour and approach.

British scientists who take part in future joint ventures with their Romanian counterparts should therefore be assured that the latter will have a lively and active part to play and that such agreements, like Dr Ceausescu's admission to the Royal Institute of Chemistry, will represent more than diplomatic lip-service to the ideals of scientific exchange.

Vera Rich

NASA plans to upgrade the role of academics

THE National Aeronautics and Space Administration is planning a major effort to increase the involvement of academic scientists in all aspects of its research and development activities.

Dr Robert Frosch, the administrator of NASA, last week signed a policy statement stating that in future academic scientists will conduct "a substantial proportion" of the basic research in all disciplines involved in the agency's programmes.

Non-NASA scientists will in consequence be closely involved in all phases of the basic research activity, from the conception and planning of a particular project, through programming and execution, to the interpretation of data and the publication of research results.

According to the policy statement, which has not yet been officially released, the agency plans to increase the use of peer evaluation of research projects at regular intervals to guarantee that a high quality is maintained throughout its activities; continuing research programmes, for example, will be reviewed by peer evaluation at least once every three years.

In the past NASA has been criticised for conducting too much of its research, particularly in the more technological areas such as space applications, on an in-house basis, with little attempt at outside evaluation. It is hoped that one effect of the new policy, which is partly a response to a directive from President Carter that all government agencies should increase their support for basic science, will help to counteract many of these criticisms.

Although no extra money has, as yet, been made available, NASA is at present working on development plans to follow the space shuttle programme, whose research and development costs peak in 1980.

Each division of the agency has been asked to prepare a plan of its future proposed activities, taking the new policy statement into account. A five-year plan for the agency based on these proposals will then be published in the fall, and this is expected to indicate ways in which, as the shuttle programme tails off in cost, support for other areas—including academic science—can be increased with no net growth in the overall budget.

One division in which the new policy is likely to have a considerable effect is the Office of Space and Terrestrial

Oceans ahoy: SEASAT-A to go aloft

SEASAT-A, a satellite designed to study the world's oceans from space, will—if all goes according to plan—be launched by the National Aeronautics and Space Administration from the Vandenberg Air Force Base in California next Monday, 26 June.

The satellite will circle the Earth 14 times a day, sending back information on surface winds and temperatures, currents, wave heights, ice conditions, ocean topography and coastal storm activity. 95% of the surface of the Earth's oceans will be covered by the satellite every 36 hours, and the data collected will be distributed to a wide range of users, including oil exploration firms, shipping companies, pollution control agencies and the weather services.

In addition to NASA, both the Canadian government's Centre for Remote Sensing and the European Space Agency will operate data-receiving stations, the former at St John's in Newfoundland, and the latter at Oakhanger, just outside London (ESA hopes eventually to establish a second station in the Canary Islands).

A number of scientific projects are being set up both to evaluate and determine the geophysical significance of the data—for example by comparing it to that being gathered by conventional surface-based techniques—and to feed it into current oceanographic studies. NASA has about a quarter of the 160 applications it had received from US scientists to conduct experiments using Seasat.

However Seasat-A is primarily a 'proof of concept' mission designed primarily to discover how effectively the microwave equipment which it carries—a scanning radiometer, a radar scatterometer, a synthetic



Artist's impression of SEASAT

aperture radar, and a radar altimeter—can provide useful scientific information for oceanographers, meteorologists and commercial sea-users. The equipment also includes a visual and infrared radiometer which will provide information to support data from the microwave sensors.

At present Seasat-A is a one-off mission with no back-up—if the launch fails, work on a new satellite will have to begin almost from scratch. However if the mission is successful, NASA hopes to launch a follow-up satellite Seasat-B in 1983 from the Space Shuttle. Eventually the plan is to develop a multi-satellite network capable of monitoring the world's oceans on a continuous, real-time basis.

applications, a major growth area in NASA's budget which covers activities such as earth resources satellites (for example the LANDSAT series), weather observation and climate research.

In the past many of such programmes have essentially been technical exercises, in which the prime objective has been to test and develop means of data acquisition, with the scientific component being added on at a relatively late stage.

As a result, academic scientists have been excluded from the early planning stages, and have had to make the best they can of the data provided to them. Under the new system, it is planned

that there will be much closer contact between the scientific community there and instrument designers, and that the former will be closely involved in helping to decide the most useful type of data—both from a scientific and a "user" point of view—that a satellite should collect.

NASA officials stress that they see the importance of such close involvement of academic scientists as much in the quality control (which it is hoped that peer review and competition for grants will bring to the programme planning process) as in helping to develop specific fields of scientific knowledge.

David Dickson

correspondence

High energy consumption or low economic growth?

SIR,—In arriving at his solution (27 April, page 744) Mr Frank Hooley, MP rejects the underlying assumption in the UK policy (and indeed in the national policy of most other countries) that there is a close relationship between the availability of energy and economic well-being. He bases his rejection on some observations about the trend in 'heat supplied' to households and to some sections of industry.

Mr Hooley is unfair to charge "nuclear power interests" with attempting to mislead the public by references to energy gaps. Atomic energy spokesmen were among the first to point out that a gap could not exist in practice (see 'Energy Prices and the Role of Nuclear Power' *Atom* August 1977). But the reason is very different from that given by Mr Hooley. It is that prices rise to bring supply and demand into balance so that industrial and domestic consumers suffer by being unable to afford the energy they need. This certainly leaves them with a gap between what they would like and what they can afford.

No one pretends that the relationship between energy consumption and economic output is simple—it is, for example, complicated by changes in the efficiency of energy use and by the substitution of one type of fuel for another, since the various fuels are not equally efficient, therm for therm, in promoting economic output. In studies of energy requirements, quality of energy is just as important as quantity. Mr Hooley overlooks this very important factor in his own cursory analysis of the trend in domestic energy use. Households have been able to maintain or even slightly reduce the 'heat' they consume (it is not all used as heat) because they have switched to energy forms of much higher quality—from coal to gas, electricity and to a lesser extent, oil. This substitution has been accompanied by a substantial increase in the primary energy consumed to meet this 'heat' requirement—because initially coal was converted to gas and electricity with some crude energy losses that reflected the greater thermodynamic efficiency of the energy finally delivered to the user. This process has been brought to a halt by the substitution of natural gas for town gas, but natural gas will not be available forever and we could see some resumption of the trend when domestic users switch back to electricity or manufactured gas.

The same trend can be discerned in some industrial uses of fuel. Mr Hooley is right to observe that the steel industry has been reducing its heat requirements per ton of steel, but its electricity requirements per ton of steel have been steadily increasing; and the general trend in industry is towards a

product mix with a higher electricity content—which is tending to offset some of the energy savings due to improvements in plant efficiency. It is however very important that these savings should continue to be made, and the Department of Energy figures incorporate a saving of 24% by the end of the century by conservation measures of one sort or another. Many experts regard this as optimistic.

Many anecdotal examples can be produced to show that we ought to be able to maintain and improve our economic wellbeing whilst holding energy consumption constant or even reducing it; and we can point to massive improvements in the efficiency of energy use that have taken place over the last century. But all the evidence is that countries are unable to sustain a pattern of falling energy consumption alongside rising output. For the world as a whole, the energy growth rate has been somewhat higher than the economic growth rate, despite all the improvements that have taken place in the efficiency of energy use, and some theoretical arguments can be advanced that at least go some way to explain this.

It would be irresponsible, on the basis of an analysis as simple as Mr Hooley's, to assume that our finite North Sea energy reserves will be sufficient to look after us until very speculative sources of energy are available at the right price and in the right quantities. Price is just as important as quantity. If the problem is seen as that of steeply rising prices then it is no solution to offer other energy sources that might turn out to be just as expensive as those they are intended to substitute.

Nuclear fired electric power is cheaper than the alternatives today—which is why electricity utilities throughout the world prefer it and why its further development attracts substantial funds. If and when the alternative energy sources reach similar stages of development and promise they too will attract much higher levels of funding. At the moment they are receiving all the funds they can absorb and are not being crowded out by the funds allocated to nuclear development.

Nuclear capacity projections have, as Mr Hooley says, been scaled down (although not by as much as the reference to figures quoted under a misunderstanding by the Royal Commission on Environmental Pollution might imply), but this has been because of an unprecedentedly prolonged economic recession and strong competition from very cheap natural gas. There is no reason to suppose that the current projections, or even higher ones, cannot be achieved. The power plant industry has, in the past, demonstrated its ability to maintain a building programme at the required rate. The existence of some surplus generating plant is no obstacle; Generating Board

spokesmen have pointed out that the surplus is more apparent than real and that base load nuclear plants ordered in the immediate future would be fully used to the economic advantage of the electricity system. Electricity economics depend upon both running costs and capital costs and it is misleading to put the spotlight upon nuclear capital costs when the running costs of the only effective alternatives are so much higher and likely to get higher still. Within the timescale that alternatives to coal, oil and gas are needed, the renewable energy sources are unlikely to constitute more than a small, unreliable and, very possibly, costly source of electricity. Their possibilities should continue to be examined because we cannot afford to overlook any possible future energy source. But we should try to keep what they have to offer in perspective. Recent correspondence in your columns have shown how often the proponents of alternatives to nuclear energy plans seem to get their facts wrong and to overstate their cases.

We can agree with the first of Mr Hooley's three final points. We should certainly not overlook the world market for power equipment of all types. Our off-shore oil and gas technology is already showing promise; and if, as seems likely, fast reactors turn out to be the preferred way of generating base load electricity we shall be glad that we remained in the forefront of the development of this technology.

It is also important not to overlook the needs of the poor countries of the world, and technologies like solar energy may have more to offer them. Their main difficulties, however, are shortages of capital and low incomes making rising energy prices hit them especially hard. If the developed countries continue to exploit low-cost nuclear energy then we are more likely to have the buoyant economies that produce surpluses of resources that can be invested in the poorer countries; and the effect of an expanding new, low-cost source of electricity will tend to keep all energy prices in check to some extent, to the benefit of rich and poor countries alike. We cannot accept, however, that there are any obvious advantages in flexibility, economics and environmental intrusion in going for small local power systems rather than inter-connected ones on the present pattern. Inter-connection provides economies of scale and the ability to deal with the failure of any single plant by the touch of a switch. The environmental advantages of separating power production from habitation seem too obvious to need spelling out.

Yours faithfully,

L. G. BROOKES

UKAEA, London, UK

news and views

Early man reviewed

from Glynn Ll. Isaac

FROM 22 to 26 May the Royal Swedish Academy of Sciences, the Nobel Foundation and the Bofors Organisation were hosts to a Nobel Symposium entitled 'Current Argument on Early Man'.* Sixteen scientists from Africa, America, Asia and Europe assembled in the town of Karlskoga, Sweden, in order to review and discuss with Swedish colleagues the state of knowledge regarding human origins and early prehistory. This symposium was one of the most broadly representative ever to be held, involving scientists from Indonesia, Kenya, the Peoples Republic of China, the USSR, and Czechoslovakia along with others from Europe, Africa and North America. The symposium was also part of a series of events commemorating the 200th anniversary of Linnaeus's death.

The symposium helped to make manifest the fact that in the course of about 100 years of hunting for 'missing links' science has acquired a fairly large number of specimens representing fossilised remains of hominids (members of the family Hominidae of which *Homo sapiens* is the only living species). We can see these specimens as a trail leading back from the more or less familiar human condition of the recent past to remote prehistoric times, in which the forms of beings represented and their ways of life are increasingly unlike any living counterparts. At the recent end of this trail fossilised human bones and archaeological remains are relatively abundant and are distributed over most of the land surface of the world. As we go back in time the geographic area over which material is known to be distributed, becomes more restricted and the record becomes relatively patchy; there are small clusters of specimens and sites which have been preserved in particular regions during particular time periods.

At present the trail of fossil hominids can be followed back with only minor gaps and breaks to about 4 million years ago. There we effectively lose the

trail, since virtually no fossilised hominoids are known anywhere from the time period between 5 and 9 Myr ago. Beyond 9 Myr back to 20 Myr science has acquired from Africa and Eurasia a rich fossil record of fossil hominoids (that is, members of the superfamily that includes humans and apes). Taxa from this time range include *Sivapithecus*, *Gigantopithecus*, *Ramapithecus* and *Dryopithecus*, and so on. However, opinion is divided as to whether any of these forms can with confidence be identified as ancestors of mankind and/or as members of the family Hominidae. Material presented at the conference, especially by David Pilbeam, of Yale University, helped to clarify issues regarding the phylogenetic interpretation of *Ramapithecus* without pretending to resolve it. Filling the 4-9 Myr gap emerged as a clear need in the advance of paleoanthropology. Most of the attention of the symposium was focused on the interpretation of the record of the past four million years. Here our present knowledge of the record seems to support a broad consensus on a matrix of fossil taxa in relation to time and geography. An approximation of this is presented below.

The discussions at the symposium suggest that there would currently be widespread agreement that the taxa represented in the fossil record are those shown on the chart and that they

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are known to be distributed in space and time as indicated. However, there was much less agreement regarding how complete the record was, and as to how the specimens and taxa could best be linked into phylogenetic schemes. For some periods the question of how many species should be recognised was keenly debated. Some scholars regard the present state of knowledge as a reasonable approximation of the 'true' evolutionary story, others regard it as only a simplistic interim version of a sequence that was probably much more complex. The symposium discussed the possibility that human ancestors by becoming culture bearing, tool-making animals at some point in their ancestry inhibited subsequent speciation. Many participants were inclined to believe that for the past million years or so there has only been one species of *Homo*, which species has been transformed, by natural selection acting over a wide area, to become modern *Homo sapiens* (a phyletic gradualist view). Others argued that while this may be the simplest and most attractive interpretation available, the 'truth' may well turn out to have been much more complex by involving, for instance, localised speciation events with subsequent 'expansions' (the punctuated equilibrium view).

The symposium discussed whether any fossils definitely attributable to *Australopithecus* (or *Paranthropus*) had yet been found in Asia. It was reported that neither the Indonesian

South Africa	East Africa	Asia	Europe
0 0.5 Myr <i>Homo sapiens</i>	<i>H. sapiens</i>	<i>H. sapiens</i>	<i>H. sapiens</i> Transition <i>H. erectus/sapiens</i>
1 Myr	<i>H. erectus</i> <i>H. erectus</i> + <i>A. boisei</i> (+ <i>A. sp.</i> ?)	<i>H. erectus</i>	
1.5 Myr <i>H. habilis</i> (<i>erectus</i>) + <i>A. robustus</i>	<i>H. habilis</i> + <i>A. boisei</i> (+ <i>A. sp.</i> ?)	<i>H. erectus</i> <i>Homo sp. ?erectus</i>	
2.0 Myr ^			
2.5 Myr <i>A. africanus</i>			
3.0 Myr v	(Newly discovered small bipedal forms with moderately large teeth and smallish brains: Laetolil and Hadar)		
3.5 Myr			
4 Myr			
A = <i>Australopithecus</i> H = <i>Homo</i> (brackets denote debated additional taxa)			

*The proceedings will be published by Pergamon Press, to appear in about 12 months.

'*Meganthropus*' nor the recently reported australopithecine teeth from China should be regarded as definite instances.

Problems of chronology continue to hamper discussions of correlation and phylogeny; we do not know with sufficient precision the relative age of the Transvaal and the East African sites, or any of the African sites and the Indonesian sites, though fresh evidence tends to confirm former indications that the earliest fossils from Java date back to between 1½ and 2 million years. The problem is particularly acute for the Middle Pleistocene (0.1–0.7 Myr). Intensified studies of biostratigraphy and a search for more widely applicable geochronometric methods, are urgently needed.

The symposium touched on the question of influences guiding evolutionary changes leading to modern human species' characteristics. Was the evolutionary differentiation of the genus *Homo* associated with the adoption of tool making and culture? What were the adaptive advantages of bipedalism? Was the enlargement of the brain in the *Homo* lineage(s) a response to the demands of culture?—or might a somewhat enlarged brain, at least at the outset, have been a preadaptation which facilitated the development of culture and technology? What, if any, were the effects of Pleistocene climatic oscillations? The symposium helped to show that we still do not know the answers to such fundamental questions. Clearly a combination of intensive hominid palaeontology, archaeology and palaeoenvironmental studies are needed for science to elucidate them better.

One participant (George Todaro, National Institutes of Health) presented aspects of the results of comparative biochemical research for consideration, summarising DNA hybridisation results. He showed that evidence continues to mount indicative of the much closer phylogenetic relationship amongst members of a set comprised of humans and the African apes, than between any members of that set and the orangutan. However, Todaro also drew attention to his studies of viral genes, which if taken at face value seem to imply the derivation of all living humans from a stock which had a long evolutionary sojourn outside Africa. This contribution dramatised the clear need for consideration of many different classes of evidence including the various kinds of molecular data, but the participants could see no simple way of integrating an hypothesis of this kind with the evidence of the fossil record as currently understood.

The symposium gave clear expression to the fact that inquiry into

human origins is no longer to be thought of as being predominantly a matter of pious studies of the bones and skulls of ancestral beings, though in fact most papers did deal with this. It was readily apparent that studies of palaeoenvironment, palaeoecology and biogeography will be crucial to the growth of our understanding. Equally, modern archaeological studies that are concerned with the economics of adaptation in early prehistory and with the development of cultural systems, are, along with palaeodemography, essential for comprehension of the dynamics of human evolution.

The first hundred years of palaeo-anthropological studies of necessity involved pioneering operations—the search for and discovery of fossils and archaeological sites followed by the description of fossils and artefacts. These operations must continue, but several researchers showed concern that the science devote some attention

to its underlying theoretical assumptions and that attempts be made to render these more explicit. In almost all circumstances it now seems important that palaeoanthropologists try to work with a set of alternative hypotheses rather than simply with one favourite interpretation. If this movement becomes established, the field could undergo an exciting phase of expanding theoretical horizons.

The study of human origins and of the biological and cultural development of the human condition is clearly a scientific endeavour of concern to all mankind. The Nobel symposium showed how much exploration, laboratory study and experimentation still remains to be done. Clearly the enterprise needs to be undertaken as an international, or better still as a pan-national effort. The symposium symbolised the growth of international interest and the mounting spirit of international collaboration. □

Linnaeus: 200 years after

by Mary Lindley

THE man who gave natural science its system of nomenclature seems to be in no danger of becoming a bore. When the bicentenary of his death was celebrated at a conference* at the Linnean Society of London on 22 and 23 May, Carl Linnaeus (1708–1778) emerged as an enthusiastic and hard-working academic with a flair for attracting patronage and a justifiable sense of self-esteem. There was good news also about his collection of plant and animal specimens, which is proving to have more to offer than taxonomists had assumed.

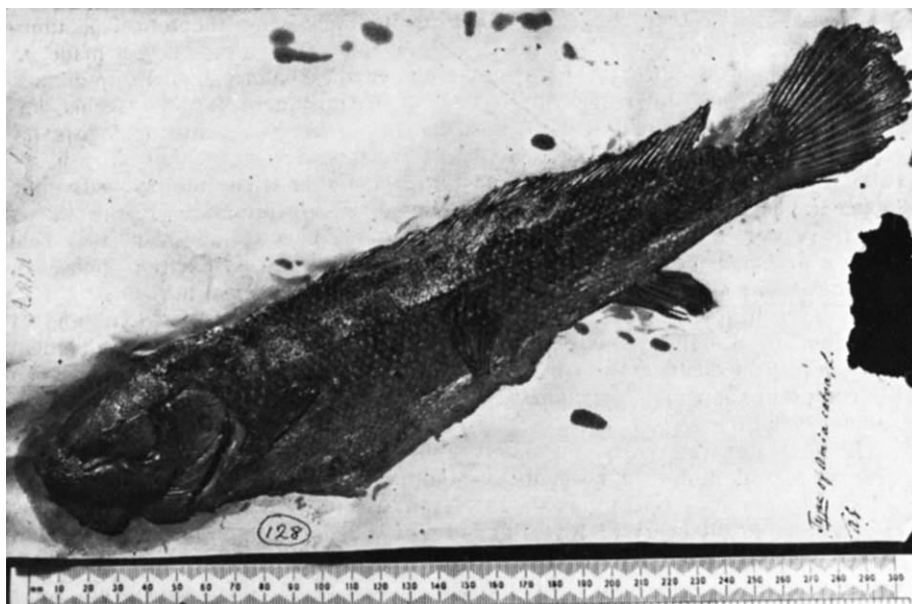
Much of what is known about Linnaeus comes from his own correspondence and publications. That literature includes his autobiography, written in the third-person and recounting his many achievements without a hint of coyness. It was quoted frequently during the conference, and especially by W. T. Stearn (Linnean Society of London) who outlined the naturalist's life and work. Linnaeus apparently acquired his preoccupation with the naming of organisms at an early age in the garden of his father's rectory at Stenbrohult in Småland, southern Sweden. In exasperation Nils Linnaeus refused to tell his son the names of any more plants until he could remember them.

In spite of his increasing knowledge

of the natural world, young Linnaeus was not an outstanding scholar and instead of following his father's example and entering the church, he studied medicine at Lund and Uppsala. As a penurious student between 1727 and 1735, sustained by the patronage of several generous academics, he began his career of travelling, collecting and writing. With his fellow student, Petrus Artedi, he made a compact to classify all the works of the Creator. When Artedi was drowned in 1735, the whole task fell to Linnaeus. Undaunted, he first edited and published Artedi's work on fishes, which appeared in 1738 as *Ichthyologia*. As A. Wheeler (British Museum (Natural History)) explained, Artedi had gathered together almost the entire knowledge of fishes then available and imposed some order upon it. Linnaeus's subsequent ichthyological work developed from this foundation.

Student travels around Sweden provided Linnaeus with the raw material for his MD thesis, which he presented at the University of Harderwijk in the Netherlands in 1741. P. C. C. Garnham (Imperial College Field Station, Ascot) has been preparing the first English translation of the thesis, in which Linnaeus refuted previous theories of the cause of malaria and replaced them with his own. On the basis of a survey of the geographical and seasonal distribution of malaria in Sweden, he concluded that the disease occurred only in marshy areas where there was

*The conference, entitled Research on Linnaeus Today—Progress and Prospects, was followed by a complementary conference in Stockholm, and the combined proceedings will be published jointly by the Linnean Society of London and the Swedish Linnean Society.



Type specimen of *Amia calva* from the Linnaean collection. The writing on the left is that of Linnaeus and on the right, that of Albert Günther, who worked on the collection in 1898–1899. The number 128 refers to the number in Günther's published list. The specimen was sent to Linnaeus on 12 April 1761 by Alexander Garden of Charles Town, South Carolina. Garden called it 'the Mud Fish'.

clay in the water. From this he developed the theory that clay particles enter the blood, eventually reaching and obstructing the smallest vessels. The body, he said, tries to unblock the blood vessels by shivering and thus expels noxious products. Although the theory is clearly fantastic, Garnham pointed out how near Linnaeus was to the correct answer when he thought of the blood, the marsh and the seasonal incidence.

Linnaeus's success as a young physician was assured when he established a reputation for treating venereal disease and for seemingly infallible diagnosis of such complaints as smallpox and malaria. The 34-year-old physician, already with an impressive list of publications, was appointed professor of medicine and botany at the University of Uppsala in 1741. In the publications that now emerged in profusion he established the system of nomenclature on which his reputation rests.

He adopted from his predecessors the practice of using a generic name with a specific epithet to form what he called a trivial name. That was a convenient alternative to the long diagnostic name which he used as a guide to the identification of a species. Thus a rush that was defined for learned purposes as *Scirpus culmo triquetro, panicula foliacea, spicula-rum squamis trifidis*, had the trivial name *Scirpus maritimus*. Trivial names first appeared in 1745 in the index of the account of his travels on the Swe-

dish islands of Öland and Gotland. He introduced them on a world scale in his *Species Plantarum* of 1753 and the tenth edition of his *Systema Naturae* of 1758—descriptive surveys, respectively, of all the plants and animals then known. J. L. Heller (University of Illinois, Urbana-Champaign), who is studying the sources of the trivial names, has found that Linnaeus adopted many from previous literature and invented many others, for example, drawing on his knowledge of classical mythology for the butterflies.

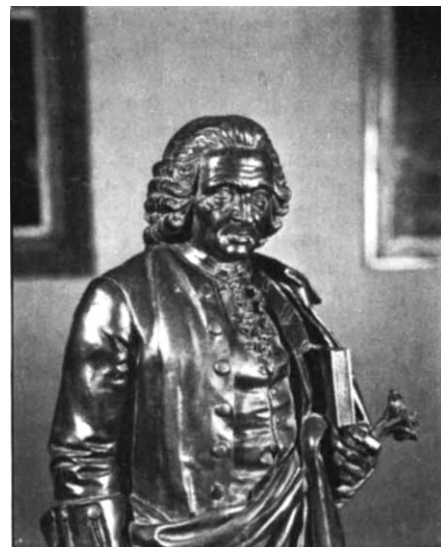
In another of his innovations, Linnaeus adopted from pharmacy the alchemical signs ♂ (Mars, iron) and ♀ (Venus, copper) and used them to signify male and female. He had a considerable interest in the sexuality of plants, on which he based his system of classification. Thus plants were grouped according to the number of stamens and pistils they possessed. According to Stearn, this system met with the disapproval of many, who felt that by discussing the sexual life of plants Linnaeus had rendered botany offensive to female modesty: hitherto it had been considered a suitable occupation for ladies. As J. L. Larson (University of California, Berkeley) pointed out, other critics had more scientific objections to Linnaeus's dependence on a single floral characteristic. At the centre of that opposition in Europe were three French thinkers, Buffon, Daubenton and Diderot, who favoured a system based on resemblances between individ-

Linnaeus's self-confidence was un-

shaken by opposition, and students flocked to Uppsala for his tuition. He was pleased to note that 23 of them subsequently became professors. Others never had the opportunity, perishing in remote and inhospitable regions in search of new material for their teacher. Linnaean apostles who survived, however, included Daniel Solander and Anders Sparrman who sailed with James Cook, round the world and south towards the Antarctic respectively.

The flow of material into Uppsala was also sustained by Linnaeus's extensive correspondence with naturalists throughout Europe. Sometimes there were formidable obstacles, such as those uncovered by C.-O. von Sydow (University of Uppsala) who has been studying the correspondence with J. G. Gmelin. As a young man, Gmelin had left his native Germany and settled in St Petersburg. As a member of Vitus Berhing's second expedition to Siberia, he brought back a rich collection which Linnaeus wished to share. Their correspondence flourished, but the successful transfer of Siberian seeds and plants across the Baltic required much perseverance. Opposition came from the superintendent of the botanic gardens in St Petersburg, J. Siegersbeck, who was one of the strongest critics of the sexual system of classification. Linnaeus dismissed Siegersbeck with the comment that he was a gardener not a botanist.

Another influential German correspondent was J. C. D. Schreber, a former student, who did much to spread his teacher's reputation in Europe, according to H. Goercke (Ludwig-Maximilians-University, Munich). Schreber translated several of Linnaeus's works, including four editions



Cast of the artist's model for the statue of Linnaeus by J. F. Kjellberg. This cast is now in the library of the Linnaean Society of London.

of *Materia Medica*—compilations of all Linnaeus's papers and all the dissertations of his students on pharmacological and pharmaceutical topics.

Materia Medica is assumed to be entirely a reflection of Linnaeus's own ideas because, in accordance with Swedish custom at that time, he wrote the dissertations, on which the students were then required to answer questions in Latin. The collected Linnaean dissertations appeared as *Amoenitates Academiae*, which covers a wide range of subjects. The zoological dissertations include some significant contributions, according to P. Smit (University of Utrecht). In one of them Linnaeus provided a simple terminology for the description of insects, introducing words such as larva and pupa. In others he dealt with the economic importance of insects. Some dissertations resembled travelogues summarising the material collected during a journey, while others were much more specific, such as one concerning the arrangement of birds' feathers.

The collection, upon which so much of Linnaeus's work depended, was bought by the English naturalist J. E. Smith in 1783 and acquired from his widow by the Linnean Society of London in 1829. By that time the specimens had suffered considerably from neglect and were somewhat depleted. The haphazard labelling and crowded arrangement of many specimens made them difficult to identify and greatly diminished their value. In 1970 when the Linnean Society built a strong room to house the collection, some of the specimens were taken into the care of experts at the British Museum (Natural History).

It has often been assumed that the collection contains none of the original specimens—type specimens—on which Linnaeus based his published description of each species. They were all thought to have been replaced or lost. This assumption, however, is mistaken. Wheeler pointed out that many of the type specimens of the fishes remain from the period when Linnaeus was relying for his descriptions on specimens rather than previous literature. Some are in collections in Stockholm and Uppsala but others, including *Amia calva* (see figure) are in London.

M. G. Fitton (British Museum (Natural History)) has found type specimens, including *Apis carbonaria*, among the Linnaean Hymenoptera after sorting through the labels and sifting sets of duplicate specimens to find those that match exactly the published description. Types have also been found among the lichens. Although Linnaeus described lichens as the 'poor trash' of nature, they occupy 317 herbarium sheets in his

collection, and P. W. James (British Museum (Natural History)) reported that about 45 specimens can be considered types.

These discoveries give the Linnaean collection renewed value for taxonomists and emphasise again the importance of the Linnean Society of London as a world centre of biological scholarship. That of course is no more than Linnaeus would have expected. □

Gamma-ray spectroscopy in astrophysics

from R. Ramaty, T. L. Kline and R. Weaver

LINE emission at gamma-ray energies is primarily the signature of nuclear processes and gamma-ray spectroscopy can be used to probe such processes at many astrophysical sites, for example supernovae and novae. Gamma-ray spectroscopy can also give information on processes of element formation. The status of gamma-ray spectroscopy was reviewed at a recent symposium.*

One of the highlights was the convincing evidence for positron annihilation radiation from the galactic centre presented by M. Levinthal (Bell Telephone) and C. MacCallum (Sandia Laboratories). The line they have observed, at 0.511 MeV, is very narrow, of width less than about 3 keV; there is also some evidence for the three photon continuum from triplet positronium annihilation. The positrons originate, most probably, either in supernova explosions, energetic particle interactions or pulsar magnetospheres. The 0.511 MeV line is the first non-transient galactic gamma-ray line to be observed with high statistical significance. With the observation of one line a variety of others are also expected, depending of course on the prevailing production mechanism. Indeed, observations with the A4 experiment on HEAO-1 reported here by J. L. Matteson (University of California, San Diego), seem to have confirmed the earlier observations by R. C. Haymes' group (Rice University) of a broad line at 4.4 MeV from the general direction of the galactic centre. This line is thought to be due to de-excitations of ^{12}C nuclei which are excited by energetic particles.

One of the most important past observations was the discovery by E. L. Chupp's group at the University of New Hampshire, of gamma-ray lines from the solar flares of 4 and 7

August, 1972. K. Hurley (Centre d'Etudes Spatiales des Rayonnements, Toulouse) showed evidence for gamma rays from the 2B flare of 22 November, 1977. If the large increase in solar activity that has taken place in the past half year is an indication of things to come, then the Sun may turn out to be a rich source of gamma ray lines during the upcoming solar maximum.

The first discovery of gamma-ray lines of cosmic origin recently reported by A. S. Jacobson (Jet Propulsion Laboratory) were described in detail at the symposium by J. Ling (JPL). These four lines at 0.41, 1.79, 2.22 and 5.95 MeV were observed in a transient event of 20-min duration with balloon-borne Ge detectors. There is no simple interpretation of these energies, but according to the model presented at the symposium by R. E. Lingenfelter (University of California, Los Angeles) the lines could be due to positron annihilation and neutron capture on hydrogen and iron at or near the surface of a neutron star.

J. Trumper (Max Planck Institute, Garching) presented observations from a series of balloon flights that showed a narrow emission feature from the X-ray binary Hercules X-1 near 56 keV with some evidence for excess emission near 110 keV. The 56 keV feature is pulsed at the period of Hercules X-1. If these features are interpreted as cyclotron emission from a strong magnetic field ($B \approx 5 \times 10^{12}$ G), then they represent the first direct measurement of the magnetic field of a neutron star. The 56 keV feature is due to the fundamental harmonic of the cyclotron radiation and the 110 keV feature is due to the first harmonic. Trumper described how these observations can be used to probe other physical conditions near the surface of the neutron star, in particular, the plasma temperature and the location and geometry of the emission process. There is evidence for long term variability since the line intensities have been observed to vary between the two balloon flights of 3 May, 1976 and 3 September, 1977.

One of the major problems of astrophysical gamma-ray spectroscopy is that of building and flying sufficiently sensitive detectors with good energy resolution. The relative merits of scintillation and Ge detectors and Compton telescopes were discussed with particular emphasis on background problems and radiation damage. There seems to be a continuing trend toward large Ge arrays, but scintillation detectors will continue to provide important contributions. Compton telescopes are expected to have great potential, mostly at high energies.

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*Held at the Goddard Space Flight Center on 28 and 29 April.

Their ability simultaneously to observe source and background regions was pointed out. Several ideas on detection techniques such as liquid argon counters and gas scintillation detectors were also presented.

The use of laboratory accelerators in support of astronomical gamma-ray spectroscopy was also discussed. Such accelerators are needed for the measurement of the nuclear cross sections that are used in the theoretical calculations of gamma ray line emission from various astrophysical sites, and for the calibration of the detectors.

The symposium ended with a review of the space flight experiments presently planned for astrophysical gamma-ray spectroscopy. High resolution Ge spectrometers will be flown on NASA's ISEE-C and HEAO-C, and on the Air Force Satellite S78-1. The HEAO-C experiment, for example, to be launched in the summer of 1979, should have an order of magnitude better sensitivity than the Bell Telephone-Sandia experiment that discovered the 0.511 MeV line from the galactic centre. Solar gamma rays will be studied with NASA's Solar Maximum Mission during the upcoming maximum of solar activity. Of particular interest is the Gamma Ray Observatory which is presently being studied by NASA as a possible new start. This observatory provides the opportunity to fly an advanced instrument which could conduct truly second generation experiments in celestial gamma-ray spectroscopy. □

Climate and energy

from A. Henderson-Sellers

It has become increasingly evident over the past few years that formulation of national and global energy policies must be based on sound knowledge of climatic regimes and must include an assessment of the impact of power production and use on climatic stability. With these aims in mind the American Meteorological Society organised their conference on Climate and Energy*. The importance of climate to both production and consumption of energy is self evident. However, the timescales of the interactions and particularly the feedback effects from energy use into climatic regimes are not easily quantified.

The need for a suitable standard data base for energy planning was underlined by H. J. Snelling and G. J. Schofield (USAF Environmental Technical Applications Centre, Illinois), who described the inapplicability of the baseline figures for the fiscal year 1975

for the recently set USA Department of Defence energy reduction programme. The apparently arbitrary choice of 1975 as a baseline for energy conservation goals has led to serious problems in many areas. The authors convincingly established the almost hopeless task of certain areas experiencing either above average winter temperatures or below average summer temperatures (in 1975) in meeting their allotted goals. Planners should be made aware of the effective and sensible use of climatic norms in any conservation policy.

Climatologists hardly need to be told about the importance of their studies in the planning of domestic and industrial fuel supply policies. However, many of us at the conference expressed disquiet over our apparent inability to communicate successfully with politicians, economists and even the media and the public. Many practising meteorologists and climatologists genuinely believe that an understanding of the vagaries of climate are now of critical global importance. L. Machta (NOAA) suggested that by the turn of the century the increase in CO₂ concentration might act as the factor which most determined global energy usage.

The problem of the globally increasing CO₂ levels has been discussed for many years now. The argument centres around the substantial absorption of terrestrial infrared radiation by CO₂ in the atmosphere. This enhances the 'greenhouse effect' and leads to increased surface temperatures. There are many reasons to doubt the results of the numerous models generated over the past decade, and climatologists would be the first to identify the problems of successful prediction. Fluctuations in climate have occurred for millennia without anthropogenic intervention and indeed the whole global system may even remain stable in spite of our interference. Clearly, climate prediction cannot meet the necessary deadlines required by politicians and economists; but, though hesitating over firm values, the delegates were deeply concerned about the ever-increasing burden of CO₂ injected into the atmosphere. Far from considering that we are crying wolf about this well-known problem, Steve Schneider (NCAR) exhorted us to continue the search for greater understanding by reference to the modern parable: "The man dropping his wallet on the pavement at night would first of all search underneath the street lamps".

A simple increase in the size of the biosphere could be used to reduce atmospheric CO₂. Growing more trees would obviously imply permanent storage of the timber to restrict the

carbon recycling. Other (more exotic) suggestions are to artificially enhance plant nutrient loadings in water bodies so that the increased aquatic biomass would rapidly utilise carbon dioxide. Carbon dioxide can be removed from chimney effluent (that is, at the industrial source) but this is at present not economically feasible without government legislation or funding.

The reverse side of the 'impact of energy on climate' debate was developed in sessions designed to consider the effects of climatic regimes and climatic change on local and global energy demand patterns. The effect of climate on energy usage is at least two-fold. Analysis of patterns of present demand can only be tackled by consideration of climatic regimes and fluctuations. Obviously any development of alternative technologies depends critically on availability and comparative usefulness of different sources. To this end an interesting study on the shores of Lake Michigan which developed equipment to monitor the relative merits of wind and solar power was described by H. Moses (Division of Biomedical and Environmental Research, US Department of Energy, Washington).

The desperate need for energy conservation and also governmental policy encouraging both insulation and use of alternative sources of power was underlined by G. A. McKay (Atmospheric Environment Service, Toronto). The need for a detailed study of domestic energy consumption and especially the impact of climatic variations upon demand patterns was described by A. Henderson-Sellers (University of Liverpool), and the problems surrounding many of the basic equations used for energy predictions were dealt with by authors such as B. H. Bailey and J. W. Scott (State University, New York).

H. Landsberg drew the meeting's attention to the fact that many planners, designers and architects seemed to be unable or unwilling to use much of the information we had been discussing. Future policies, and particularly long range forecasting of both energy and climate, must be inextricably linked. □

Realities of fusion power

from R. Carruthers

A SUCCESSFUL fusion reactor calls for much more than the solution of plasma physics problems. This has long been recognised by the American Nuclear Society who have sponsored an important series of 'Topical Meetings on the Technology of Controlled Nuclear

*Held in Asheville, North Carolina, 8-12 May, 1978.

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Fusion.' The third of the series—'The Commercialisation of Fusion Power'—was held recently* and served to emphasise the growing disparity between the fusion programme of the USA, the rapidly expanding activity in Japan and the programme in Europe (EEC).

The tone of the meeting was set in the keynote address by J. W. Mayo (US Office of Energy Technology) who emphasised government commitment to the fusion programme and reminded participants that "... we are in the business of creating a user-oriented programme. We are creating a product."

The participation of many representatives of the electric utilities, industry and consultants showed that the ultimate users of fusion power were already becoming involved in the shaping of the future programme. This revealed a problem which recurred throughout the meeting, and particularly in a panel session chaired by R. L. Hirsch (Exxon Corporation): with long term fusion plans indicating about 30 years to a 'commercial' prototype, how could the substantial continuing support be justified and industrial involvement encouraged and maintained? To counter this, R. Carruthers (Culham Laboratory) questioned whether the 30-year plans were the most cost effective route to fusion power and suggested a higher risk, shorter term objective to focus attention on critical, reactor-relevant problems. Representative Manuel Lujan Jr. (New Mexico) called for the development of a much more effective fusion lobby and said that Congress would be interested in considering requests for higher funding for more aggressive, higher risk proposals.

It is no longer sufficient to show that a technological *tour-de-force* could produce a reactor concept for a particular method of plasma confinement. The realities of the concept must be evaluated and more than a third of the papers dealt with operation and maintenance, safety, and fusion reactor engineering and technology. The toroidal geometry of tokamaks presents many operational problems. Five reactor concepts were compared by G. M. Fuller and H. S. Zahn (McDonnell Douglas Astronautics Co-East) and showed the poor availability and consequent high electricity cost which could result from a design which was too complicated for rapid servicing. The simplification of tokamak concepts was represented by the latest study from the group at the University of Wisconsin (Madison). Their

NUWMAK proposal has a simplified magnet structure to give better access; operation at a lower temperature eases the material problems and leads to a cooling system very similar to fission's BWR, and a novel, though perhaps questionable, scheme is proposed to eliminate the magnetic divertor.

Linear confinement systems are geometrically more attractive but their reactor potential has always been questioned because of the high end losses. Many concepts are being studied to reduce these end losses, particularly the Tandem Mirror and the Reversed Field Mirror (G. A. Carlson *et al.*, Lawrence Livermore Laboratory) but these may still not be adequate to meet the needs of an economic fusion reactor. A similar problem faces the concepts for inertial confinement driven by lasers, electron or ion beams—the requirements for high efficiency drivers and a low proportion of recirculating power are technologically difficult to meet. These confinement concepts which may have difficulty in achieving the stringent requirements of a fusion power reactor could be very economic sources of neutrons and many studies were reported on fusion/fission hybrids. The fusion neutrons would be used to convert a fertile blanket of ^{238}U or ^{232}Th into fuel for a thermal fission reactor, one hybrid having the potential to fuel five or more LWRs.

Plasma engineering is a relatively new subject of growing importance. The plasma in a fusion reactor has to be 'controlled' to maintain the required parameters, compared with the self-generated configurations in most present day plasma physics experiments. A. T. Mense *et al.* (Oak Ridge National Laboratory) in a study of heating and refuelling a reactor showed the importance of this type of work which could substantially modify some of the simple assumptions used in many reactor studies.

The extensive work reported at Santa Fe was difficult to reconcile with the suggested long time scale to a power reactor. The technological problems are indeed challenging but a comparison with the pace of developments of fission is unavoidable—there were no Topical Meetings on Fission 30 years before Calder Hall! □

Sliding microtubules

from Michael Spencer

A RECENT meeting of the British Biophysical Society on Cell Motility (held at Queen Elizabeth College in December 1977; abstracts from M. E. J. Holwill) brought out very clearly that although there are great superficial differences between the

various mechanisms of motility, there are cases where quite different proteins seem to generate movement in remarkably similar ways. The most interesting comparison is between the actomyosin system of striated muscle and the microtubule-dynein combination of cilia and non-bacterial flagella; they are both thought to work by the attachment, bending and detachment of cross-bridges, the bridges carrying the sites of ATPase activity. Some recent papers in the *Journal of Cell Biology* illustrate progress in studying the latter system.

At the light-microscopic level Robert Rikmenspoel (*J. Cell Biol.* **76**, 310; 1978) has analysed high-speed cinemicrographs of sea urchin sperm flagella, including two interesting cases of flagella starting from rest. These flagella were initially almost straight and developed a full and apparently normal wave within the time of one period; it did not seem to propagate down from one end, and certainly did not build up slowly like a resonance oscillator. At all locations on the flagella the transverse motion and the curvature varied sinusoidally with time, though the wave shape at any given time was not sinusoidal. Rikmenspoel concludes that the force-producing mechanism should also vary sinusoidally with time. Data of this kind will be useful in evaluating the various theoretical models that have been proposed.

Meanwhile, down among the molecules Warner and Mitchell (*J. Cell Biol.* **76**, 261; 1978) have been studying *Tetrahymena* cilia in the electron microscope after demembration and treatment with ATP (Summers & Gibbons, *Proc. natn. Acad. Sci. U.S.A.* **68**, 3092; 1971; *J. Cell Biol.* **58**, 618; 1973). This causes some cilia to shoot out like telescopic radio aerials, stripping off many of their cross-bridges in the process; others, however, come apart more gently so that the dynein arms can be seen sticking out from the microtubules at a well-defined angle. Sale and Satir (*Proc. natn. Acad. Sci. U.S.A.* **74**, 2045; 1977) had shown that in this system each microtubule doublet slides relative to its neighbours in a single direction, presumably through the dynein arms of one doublet 'walking' along another; the polarity of force generation is from base to tip of the cilium. They also observed that the arms were tilted towards the base, in both intact and disrupted axonemes. Thus the arms are positioned like the oars of a rowing boat before the active stroke.

Warner and Mitchell have taken this somewhat further by presenting

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*Held on 9–11 May in Santa Fe, New Mexico.

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evidence that in the absence of ATP the arms are not attached to the adjacent doublet, while in its presence they are attached by the terminal member of the 3-4 subunits that compose each arm. In both cases the arms point towards the base of the axoneme, making an angle of $32^\circ \pm 4^\circ$ with the perpendicular. This is disappointing in that there is still no evidence for a second orientation, as one would expect in a cyclic motion; in striated muscle there is some X-ray and electron microscopic evidence for two orientations, and theories have been constructed to explain them (Eisenberg & Hill *Prog. Biophys. molec. Biol.* **33**, 55; 1978). Warner and Mitchell do observe two slightly different appearances of dynein in their micrographs, but they think this is only a minor variation between the inner and outer rows of arms.

There are other differences from the situation in muscle. First, the cross-bridge attachment in muscle is believed to occur with the myosin S-1 at right angles to the actin filament, so that the active stroke involves bending away from the perpendicular; in *Tetrahymena* the opposite is postulated, though *Chlamydomonas* may be different (Allen & Borisy *J. molec. Biol.* **90**, 381; 1974). Second, in muscle the binding of ATP causes detachment of cross-bridges, and depletion of ATP gives the attached state of rigor—again opposite to what is suggested by Warner and Mitchell for cilia, though not at variance with some earlier results of Gibbons and Gibbons (*J. Cell Biol.* **63**, 970; 1974).

There is still a fascinating problem to be solved in working out how a sliding motion could drive the characteristic beat of a cilium. Witman *et al.* (*J. Cell Biol.* **76**, 729; 1978) have studied mutants of *Chlamydomonas* that lack either a protein forming the spokes between central and outer doublets, or three proteins making up the central tubule-central sheath complex. None of the mutants would beat when isolated axonemes were reactivated with ATP, yet all of them showed relative sliding of outer doublets when treated with both trypsin and ATP. The findings support the hypothesis of Warner and Satir (*J. Cell Biol.* **63**, 35; 1974) that radial spoke-central sheath interactions are essential to the conversion of sliding into bending.

All the differences of detail between muscles and cilia do not obscure the fact that striking similarities exist. Since nobody has so far demonstrated exactly what happens during the active stroke of any motile system, progress in understanding one may shed a great deal of light on the others; frustrated muscle workers who dream of cry-

stallising different states of the myosin cross-bridges can usefully consult their ciliary colleagues, and *vice versa*. Brave heretics who disbelieve in cross-bridge movement may perhaps remain undaunted; but after recent extraordinary revelations about the workings of bacterial flagella (summarised in *Nature* **263**, 370; 1976; **267**, 15; 1977), no mechanical analogue seems too far-fetched. □

How cells cycle

from Richard Braun

CYTOLOGICAL studies on individual cells and biochemical experiments on synchronised cells have accumulated a considerable body of evidence about what happens when in the life of a cell. But many important causal interactions in the life of a cell were elusive until geneticists came up with the specific cell cycle mutants.

At a recent workshop* L. Hartwell (University of Washington, Seattle), who opened up this field in yeast, discussed two of his recent mutants, *cdc4* and *cdc28*, which seem to code for different subunits of the spindle pole body, which itself is essential for orienting the spindle apparatus. It is particularly noteworthy that non-growing yeast cells are arrested just before spindle pole body duplication and this is also the time point at which mating factor controls cell cycle progression. It therefore seems that at least in budding yeast, spindle pole body duplication is an important cell cycle control event necessary for the initiation of DNA replication. In a fission yeast, *Schizosaccharomyces pombe*, very different cell cycle mutants have been characterised (P. Thuriaux, Bern; P. Nurse & K. Nasmyth, University of Edinburgh). One mutant is DNA ligase deficient, while two other classes of mutants, *wee1* and *wee2*, have an abnormally small size at division. Obviously the cell mass (or size) gauging device is defective in these mutants. Although the products of the two *wee* genes have not been isolated, they may conceivably act as an inhibitor/promoter pair of mitosis.

An interesting cell cycle dissection technique involving fusion and premature chromosome condensation (PCC) was discussed by W. Hittelman (University of Texas, Houston). Fusion of a mitotic cell with an interphase cell leads to PCC in the new cells. Upon staining, the chromosomes of the former interphase cell will have a characteristic appearance depending on where it had been in interphase when the cells were fused. The phenomenon

of PCC is therefore a useful diagnostic tool and can with a reasonable degree of accuracy distinguish up to six temporal segments of the G1 phase in mammalian cells. Applying this technique it was shown that normal human cells are arrested under non-growth conditions in very early G1, whereas tumour cells stop in late G1. In leukaemic patients PCC may allow prediction of the duration of a remission: shortly before a relapse, lymphocytes are often in later G1 stages than during a stable remission. S. Shall (University of Sussex) suggested that the re-entry into a division cycle may be quite differently controlled in unicellular and multicellular organisms. In single cells the available energy may be decisive, while in multicellular organisms a signal has to come to the individual cell telling it to prepare for division. C. Petzelt (University of Heidelberg) has recently found an interesting Ca-dependent ATPase which seems to be specific for the spindle of mitotic cells and may lead to microtubule condensation. It may be of general significance that the last phase of DNA replication in *Physarum* as reported by F. Haugli (University of Tromsø), namely the ligation to chromosome-size molecules, may be very slow and extend far into the G2 phase. So G2 could be a low key continuation of S. A peculiarity of *Physarum*, which does not invalidate this conclusion, is the observation that genes for rRNA replicate primarily in G2. With respect to the other end of the S-phase, F. Grummt (University of München) presented evidence suggesting that diadenosine-tetraphosphate (Ap_4A) may act as an initiator of DNA replication in animal cells.

The unicellular algae are a good model system to study biochemical events of the cell cycle, as reported by P. John (University of Belfast): light-dark cycles induce good synchrony. For meaningful results, however, cells need to be studied in a turbidostat in order to prevent perturbations due to increased light absorption by the expanding cell mass. From this study and others reported at the workshop, it seems that in general less than 5% of proteins are synthesised at discrete steps in cell cycles, while the bulk of proteins rather increases continuously from one mitosis to the next. The minority group, which is of course of particular interest with respect to cell cycle control, contains enzymes functioning in DNA replication and also the histones. As discussed by H. Matthews (Portsmouth Polytechnic) not only is the actual synthesis of histones periodic, but also their modification.

*The Fourth European Cell Cycle Workshop, held on 17-19 April in Bern.

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review articles: parasitology supplement

Epidemiology and ecology of leishmaniasis in Latin-America

R. Lainson* & J. J. Shaw*

Of the diseases caused by protozoal parasites, leishmaniasis is probably second in importance only to malaria. Chemotherapeutic drugs are toxic, expensive and not 100% effective. This, and the absence of any non-living vaccine against the disease, means that control depends on eliminating either reservoirs or insect vectors, or both. Recently, a greatly increased knowledge of the Leishmania species involved, and of their natural hosts, has helped to define the nature and extent of the problem.

LEISHMANIASIS is caused by protozoan parasites belonging to the genus *Leishmania* (Phylum-Protozoa: Order-Kinetoplastida: Family-Trypanosomatidae) whose life cycles are completed in two different hosts, a vertebrate and an insect. With the trypanosomes they are often loosely referred to as the 'haemoflagellates'.

Vertebrate hosts are principally confined to mammals, although some *Leishmania* species are found in reptiles: no infections have yet been recorded, in nature, in any other vertebrate groups. There is usually a well balanced host-parasite relationship in the natural vertebrate host, and the parasites are scattered, in small numbers, in macrophages of the skin, viscera or blood, where they produce few or no pathological effects. In unusual hosts, including man, however, there may be violent host-cell reaction to the parasite which results in skin lesions (cutaneous and mucocutaneous leishmaniasis) or severe pathological changes in the internal organs ('kala azar', and other forms of visceral leishmaniasis). Within the macrophages the organism is in the amastigote form (Fig. 1)—a round to oval body, about $1.5\text{--}3.0 \times 3.0\text{--}6.5\text{ }\mu\text{m}$ in size, depending on the species. The cytoplasm stains a pale blue with Romanovsky stains, and the single nucleus and rod-shaped kinetoplast stains reddish-purple: there is no free flagellum, but a rudimentary one is present in the flagellar pocket, a small invagination of the parasite's surface. Reproduction is by binary fission.

All known vectors of the leishmaniae are phlebotomine 'sandflies' (Fig. 2) (Diptera: Psychodidae: Phlebotomidae) which have a wide distribution in tropical and subtropical regions of both the Eastern and Western Hemispheres. Amastigotes are taken up from the skin or peripheral blood when the female sandfly feeds on an infected host. During the first 72 h the amastigotes elongate, within the stomach

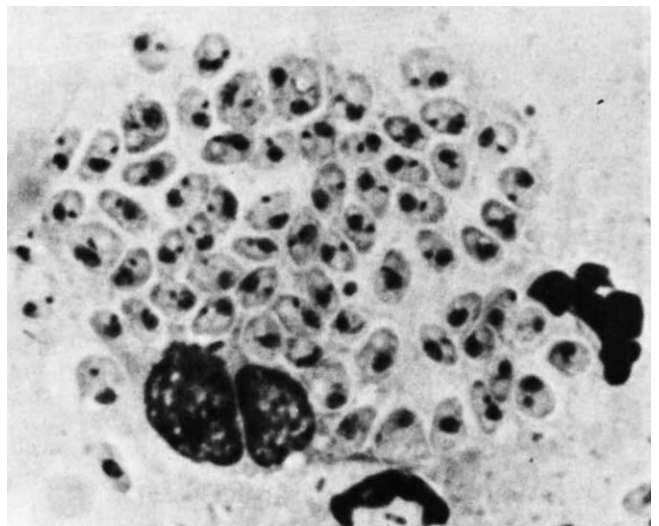


Fig. 1 Amastigotes of *Leishmania mexicana amazonensis* in a smear from a skin lesion of an experimentally infected hamster. Giemsa, $\times 1,700$.

contents, and the rudimentary flagellum grows out into a long whip-like structure. The resulting promastigotes (Fig. 3) undergo extensive longitudinal binary fission: they vary from 16.0 to $40.0\text{ }\mu\text{m}$ long, by 1.5 to $3.0\text{ }\mu\text{m}$ wide. In the life cycle of most leishmaniae (certainly all those known from mammals) the parasites ultimately migrate to the anterior parts of the sandfly gut, where they become attached to the cardinal wall, in vast numbers, by the flagellum. Further migration of small, free forms to the pharynx and proboscis leads to ultimate inoculation of some promastigotes into the skin of a further vertebrate host when the sandfly takes another blood meal. The time

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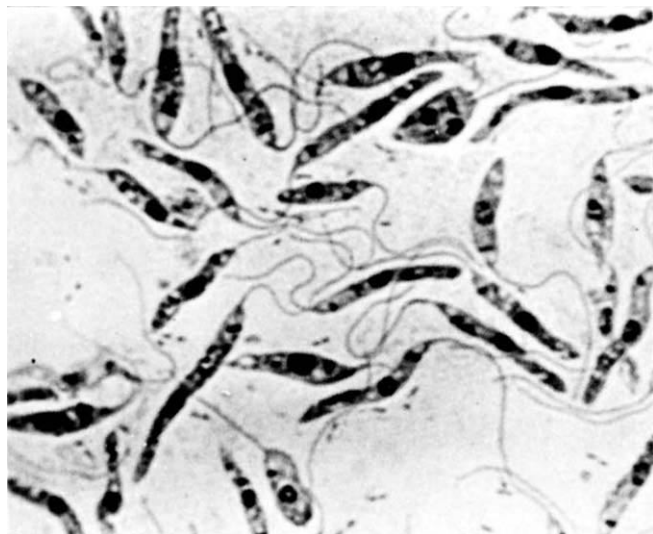


Fig. 2 Promastigotes of *L. m. amazonensis* from the midgut of a naturally infected sandfly, *Lutzomyia flaviscutellata*. Pará State, Brazil. Giemsa stain, $\times 1,700$.

taken for the parasite to undergo its complete cycle in the insect vector depends on both the species of *Leishmania* involved, and local conditions (for example, temperature). It may be transmitted in as little as 4 days after the infective blood meal (subspecies of *L. mexicana*), or only after three consecutive blood meals (*L. infantum*).

Medically, leishmaniasis can reasonably be regarded as second in importance only to malaria among the protozoal diseases of man. Kala azar ('black sickness') is almost always fatal, unless adequately treated, and has exacted an enormous toll of human life, particularly in India where serious new outbreaks are now occurring: other forms of the visceral disease have also caused great loss of life in the Mediterranean countries, East Africa and many Latin-American countries, in particular Brazil. While it is less dramatic, cutaneous leishmaniasis, in both the New and the Old World, can be very disfiguring: this is especially so for the highly mutilating mucocutaneous leishmaniasis of South America, which may destroy nasal and pharyngeal tissues (Fig. 4), and the incurable 'diffuse cutaneous leishmaniasis' in both the Old and the New World (Fig. 5) which is found in patients who have defective cell-mediated responses to leishmanial antigens. The disease is particularly important, economically, in those developing countries already wrestling with other public health problems. Cutaneous and mucocutaneous leishmaniasis are very common problems among labour forces engaged in the development of heavily forested areas, such as in the Amazon region of Brazil.

Classification of the American leishmaniae

The first recorded autochthonous cases of cutaneous leishmaniasis in the Americas were independently registered by Lindenberg¹ and Carini and Paranhos², in patients from south Brazil, in 1909; and the name *Leishmania braziliensis* was given to the causative organism from a further case in Minas Gerais, Brazil, by Vianna³ in 1911. For many ensuing years the view persisted that all forms of American cutaneous and mucocutaneous leishmaniasis were due to this same parasite, throughout all the South American continent; an attitude which undoubtedly has seriously hampered our progress in elucidating the ecology and epidemiology of the different forms of the human disease. American visceral leishmaniasis of man was first recorded⁴ in 1913, in Paraguay, and has since been found in almost all Latin-American countries. A similarly extreme view long prevailed that all forms of visceral leishmaniasis were due to *Leishmania donovani* (Indian 'kala azar') whether from India, Africa, Europe or Latin-America, and this once again

seriously hindered our appreciation of the true epidemiological situation in the Americas. Workers in Brazil⁵ eventually showed the disease in that country to be a zoonosis, with the source of infection for man in dogs and foxes: quite different, therefore, from that in India, where the parasite is transmitted only from man to man and the disease is thus an anthroponosis. In spite of this, and the new name of *Leishmania chagasi* given to the parasite in 1937⁶, visceral leishmaniasis of the New World is still erroneously referred to by many as kala azar and the causative organism as *L. donovani*. The words kala azar are Hindu for black sickness, from the strange earthy-grey colour of persons suffering from the Indian disease: it is not applicable, therefore, to the clinical picture seen in Latin-America, where it is more correct to refer to the disease as American visceral leishmaniasis due to *L. chagasi*.

In spite of misgivings on the part of many notable parasitologists, new specific or subspecific names began to appear for a number of leishmaniae, based on steadily increasing biological and biochemical criteria⁷⁻¹⁰. In recent years, for example, it has become apparent that both *L. braziliensis* and *L. mexicana* are specific names embodying numerous subspecies, some of which still need to be adequately defined. Some subspecies of these parasites infect man only rarely, because the vectors are not particularly anthropophilic: other leishmaniae probably exist which, like *L. enriettii* and subspecies of *L. hertigi*, are either incapable of infecting man, or seldom if ever have the opportunity because of lack of contact between man and the sandfly vectors. Table 1 summarises the leishmanial parasites that are now generally recognised from the South American continent¹¹, and there are doubtless many more to be described.

Our relatively recent awareness of a number of different subspecies of *Leishmania* infecting man is of considerable importance to the clinician, and prognosis may be markedly influenced by correct identification of the parasites involved. There is the sombre fact, for example, that although infection with *L. mexicana amazonensis* is relatively rare in man, it does remain the only known cause of Amazonian diffuse cutaneous leishmaniasis in Brazil: this is incurable, highly disfiguring, and has been shown to develop in as many as 6 out of 20 (30%) patients with *L. m. amazonensis* infection so far studied in our laboratory. *L. braziliensis braziliensis* infection is often associated with metastases to the naso-pharyngeal mucosae, with very considerable mutilation: on the other hand, there is no evidence of such involvement following infection with the related parasite *L. braziliensis guyanensis*. Correct identification of the parasites in early skin lesions will do much to orientate treatment, prognosis and clinical follow-up.

Finally, recovery from any leishmanial skin lesion was generally thought to impart a firm and life-long immunity to reinfection. Whilst this may hold good for homologous parasite challenge, there is now known to be a surprising lack of cross-immunity between at least five of the subspecies of *Leishmania* infecting man in the Americas¹² (*L. m. mexicana*, *L. m. amazonensis*, *L. b. braziliensis*, *L. b. guyanensis* and *L. b. panamensis*). The chances of repeated infection are thus much higher than previously supposed,

Fig. 3 A sandfly, *Lutzomyia* sp., biting man. $\times 10$.



Table 1 Leishmanial parasites now recognised in South and Central America

Parasite	Disease in man	Recorded mammalian hosts	Recorded sandfly hosts	Known geographical distribution
<i>Leishmania braziliensis braziliensis</i>	Cutaneous and mucocutaneous leishmaniasis ('úlceras de Baurú', 'espundia')	Forest rodents: <i>Oryzomys</i> , <i>Proechimys</i> and <i>Akodon</i> : man (accidental host)	<i>Lutzomyia intermedia</i> , <i>Lu. pessoai</i> , <i>Psychodopygus wellcomei</i> *	Brazil (type locality, Minas Gerais)
<i>Leishmania braziliensis guyanensis</i>	Cutaneous leishmaniasis ('pian-bois', 'bosch-yaws', 'forest-yaws')	Wild reservoir unknown; man (accidental host)	<i>Lutzomyia umbratilis</i> *	Guyana, Surinam, French Guyana; in Brazil, north Pará, Amapá, and into Roraima and Amazonas
<i>Leishmania braziliensis panamensis</i>	Cutaneous leishmaniasis	The sloths <i>Choleopus</i> (major host) and <i>Bradypus</i> ; the procyonids <i>Basicyon</i> , <i>Nasua</i> and <i>Potos</i> (secondary hosts). The primates (secondary hosts) and man (accidental host)	<i>Lutzomyia trapidoi</i> *, <i>Lu. ylephiletor</i> , <i>Lu. gomezi</i> and <i>Psychodopygus panamensis</i>	Panama and probably into neighbouring Costa Rica and Colombia
<i>Leishmania peruviana</i>	Cutaneous leishmaniasis ('uta')	Dog (<i>Canis familiaris</i>); wild reservoir unknown	None. <i>Lutzomyia verrucarum</i> and <i>Lu. peruensis</i> strongly suspected	Western slopes of the Peruvian Andes and the Argentinian highlands
<i>Leishmania mexicana mexicana</i>	Cutaneous leishmaniasis; rarely diffuse cutaneous leishmaniasis ('chiclero's ulcer', 'Baysore')	Forest rodents: <i>Ototylomys</i> , <i>Heteromys</i> , <i>Nyctomys</i> and <i>Sigmodon</i> . Man (accidental host)	<i>Lutzomyia olmeca olmeca</i> *	Belize, Guatemala, Yucatan Peninsula
<i>Leishmania mexicana amazonensis</i>	Cutaneous and diffuse cutaneous leishmaniasis	Forest rodents: <i>Proechimys</i> , <i>Oryzomys</i> (primary hosts); <i>Neacomys</i> and <i>Dasyprocta</i> , marsupials: <i>Marmosa</i> , <i>Metachirus</i> : carnivores: fox, <i>Cerdocyon thous</i> (all secondary hosts). Man (accidental host)	<i>Lutzomyia flaviscutellata</i> *	The Amazon region of Brazil and probably of neighbouring countries
<i>Leishmania mexicana pifanoi</i>	Diffuse cutaneous leishmaniasis, almost certainly simple cutaneous leishmaniasis	Forest rodents: to date, only <i>Heteromys</i> . Man (accidental host)	<i>Lutzomyia flaviscutellata</i>	Venezuela
<i>Leishmania mexicana</i> subspecie	Not yet recorded	Forest rodents: <i>Oryzomys</i> (primary host), <i>Proechimys</i> , <i>Dasyprocta</i> . Marsupials: <i>Marmosa</i> (all secondary hosts)	None. <i>Lutzomyia olmeca bicolor</i> strongly suspected	Sassardi region of Panama
<i>Leishmania mexicana</i> subspecie	Not yet recorded	Forest rodents: <i>Oryzomys</i> (primary host), <i>Proechimys</i> , <i>Heteromys</i> . Marsupials: <i>Marmosa</i> , <i>Caluromys</i> (all secondary hosts)	<i>Lutzomyia flaviscutellata</i>	Island of Trinidad, WI
<i>Leishmania enriettii</i>	Not yet recorded	Domestic guinea pig (<i>Cavia porcellus</i>): not yet found in any wild animal.	None	Paraná State, Brazil
<i>Leishmania hertigi hertigi</i>	Not yet recorded	Porcupine, <i>Coendou rothschildi</i>	None	Panama, Costa Rica
<i>Leishmania hertigi deanei</i>	Not yet recorded	Procupine, <i>Coendou prehensilis</i> and other species of this genus	None	States of Piauí and Pará, Brazil
<i>Leishmania chagasi</i>	American visceral leishmaniasis	Foxes <i>Lycalopex</i> and <i>Cerdocyon</i> ; dog (<i>Canis familiaris</i>) (secondary hosts?); man (accidental host)	None. Epidemiological evidence all points to <i>Lutzomyia longipalpis</i>	Most of the dryer and poorly forested regions of the South American continent

*Incriminated as the principal vector.

especially in persons who travel widely in the South American continent.

Ecology and epidemiology of New World leishmaniasis

As far as is known, all forms of American leishmaniasis in man are zoonoses: that is, they are derived from a source in wild and/or domestic animals and transmitted to man by the sandfly vectors, when he intrudes into the enzootic areas. In most cases the reservoir of infection is in sandflies and mammals of the dense, tropical rain-forests. Some

species of sandflies, however, have adapted to domestic and peridomestic situations, especially in the drier regions. This has led to the disease occurring in man in nonforested areas with the dog becoming an important source of infection ('uta' and American visceral leishmaniasis).

Recent studies indicate that there is a natural limitation of individual species or subspecies of *Leishmania* to specific sandflies, even when there is a wide variety of these insects in the same general environment. The precise reasons for this are still not completely clear; but it may be due to an intimate vector/mammalian host contact in restricted

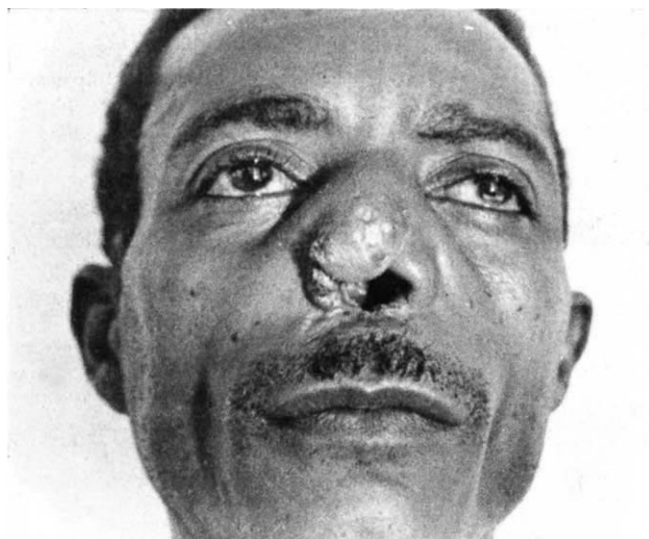


Fig. 4 Mucocutaneous leishmaniasis due to *Leishmania braziliensis*. Lesions also extend to the soft palate.

ecological foci, which could then lead to obligatory development of certain leishmaniae in particular sandfly species. For example, extensive examination of many sandfly species of the northern Amazon region of Brazil has revealed no vector of *L. m. amazonensis* other than *Lutzomyia flaviscutellata*¹³; the transmission of *L. b. braziliensis* in the same or similar forests seems limited to *Psychodopygus wellcomei*¹⁴; in north Brazil and the Guyanas the transmission of *L. b. guyanensis* seems to be only by *Lutzomyia umbratilis*^{15,16}; while the vector of *L. m. mexicana* in northern Central America seems only to be *Lutzomyia olmeca olmeca*^{17,18}. In all these regions there are abundant other sandfly species attracted to both the wild mammalian hosts and man.

Studies along the 3,000 km-long Transamazon highway, recently constructed in the Amazon region of Brazil, have indicated different anthropophilic sandfly species to predominate in particular areas¹⁹. It may be that some of these will be shown to be specific vectors of hitherto undescribed leishmaniae, which could in future infect newly arrived colonists in the previously uninhabited areas. Lengthy comparison of isolates from sandflies, wild animals and man will be necessary to bear out this hypothesis.

Subspecies of *Leishmania mexicana*

The subspecies of *Leishmania mexicana* are parasites of silvatic mammals, in particular rodents and marsupials, in which they are relatively harmless apart from the occasional production of mild skin lesions. Their vectors belong to a group of sandflies which are not highly attracted to man. As a result, although the incidence of *L. m. amazonensis* may be as high as 20% in some forest rodents of Brazil⁸, human infection remains rare²⁰. Transmission to man is limited even further by the essentially night-time feeding of the vector, *Lu. flaviscutellata*, and the insect's common occurrence in the low and wet 'igapo' forest which is little used by man.

The vector of the closely related parasite *L. m. mexicana*, in northern Central America, *Lu. olmeca olmeca*, is similarly highly attracted to the wild rodent hosts but relatively uninterested in man. For this reason 'chiclero's ulcer' would doubtless be a relatively rare disease, were it not for the fact that the 'chicleros' are obliged to live for almost 6 months of the year in the high forest, while collecting the chewing-gum latex from the chicle trees. This period unhappily coincides with the rainy season and, therefore, with the maximum population of *Lu. o. olmeca*.

L. m. pifanoi is clearly closely related to *L. m. amazonensis* and has till now only been recorded in a few cases of diffuse cutaneous leishmaniasis (DCL) of man, in Venezuela. Cases of simple leishmaniasis due to the same parasite almost certainly exist, but have presumably not yet been noted because of their rarity and inconspicuous nature compared with the very obvious DCL.

The subspecies of *L. mexicana* discovered in rodents and marsupials in Panama²¹ and Trinidad²² have not yet been recorded as responsible for any form of human leishmaniasis. The very poor vector/man contact again probably accounts for this: in Panama rare infections of man are likely to be overlooked among the large number of cases due to *L. braziliensis panamensis*.

Subspecies of *L. braziliensis*

An extensive classification of *L. braziliensis* has till now been greatly hampered by the extreme difficulty with which many of them are cultivated in either blood-agar media or in laboratory animals^{7,8}; this contrasts markedly with the relative ease in growing subspecies of *L. mexicana*. As a result, only three distinct subspecies of *L. braziliensis* have been clearly differentiated, although we are acutely aware of a number of others which need to be categorised and named¹¹: these include important agents of cutaneous and/or mucocutaneous leishmaniasis of man in parts of Brazil and neighbouring South American countries. Biologically, all known subspecies of *L. braziliensis* can readily be distinguished from those of *L. mexicana* by their pattern of development in the sandfly²³. In addition to growth in the midgut and foregut of the vector, *L. braziliensis* undergoes development in the hindgut, where the parasites are predominantly attached to the wall of the pylorus. Such development is absent in the life cycle of *L. mexicana*, which develops only in the mid and fore gut.

The vectors of the three recognised subspecies of *L. braziliensis* are much more anthropophilic than those of *L. mexicana*, and there is a correspondingly higher incidence of human leishmaniasis due to the former parasites. *Psychodopygus wellcomei*, the main vector of *L. b. braziliensis* in the Serra dos Carajás region of north Brazil, attacks man viciously, not only at night but also in broad daylight—a factor which greatly increases the risk of human infection: the natural resting-site of this insect in the forest remains a complete mystery. *Lutzomyia umbratilis*, the vector of 'pian-bois' due to *L. b. guyanensis* in north Brazil and the Guyanas, is somewhat less attracted to man: the daytime resting sites of this sandfly are on the trunks of larger trees, where it occurs in large numbers during the

Fig. 5 Diffuse cutaneous leishmaniasis (DCL) due to *L. m. amazonensis*. Thirty-year-old infection. Para State, Brazil.





Fig. 6 American visceral leishmaniasis due to *Leishmania chagasi*, Ceará State, Brazil, showing typical enlargement of liver and spleen.

wet season. *Lu. umbratilis* also bites man by night and day, particularly when disturbed from the tree trunks: forest workers clearing land for plantations or other purposes are particularly at risk and, as the incident of infection in the vector population may be as high as 7% in some areas, few persons penetrating the forest escape infection. In Panama, and probably neighbouring Costa Rica, the principal mammalian host of *L. b. panamensis* is the sloth *Choloepus hoffmanni*²⁴ and the main vector *Lutzomyia trapidoi*²⁵. This association of vertebrate and insect hosts in the forest canopy would not at first sight seem to offer much risk of infection for man. At certain times, however, the sandfly vector can be found abundantly on the lower parts of the tree trunks or ground vegetation, whence it is readily attracted to man²⁶.

Leishmania peruviana (Peruvian and Argentinian 'uta')

No one who has worked on cutaneous leishmaniasis in the dense, humid forests of the New World can fail to be impressed by the stark contrast presented by the endemic areas of 'uta', caused by *L. peruviana*, on the barren slopes of the Peruvian Andes and the Argentinian highlands. Until now, the only known animal reservoir is the domestic dog²⁷, although the possibility of a primary source of infection in some wild animal(s) has been far from adequately investigated. Transmission seems to be domiciliary: the sandfly vector remains to be incriminated, although both *Lutzomyia verrucarum* and *Lu. peruensis* are highly suspect because of their coincidental distribution in and near the houses of infected persons²⁸. The epidemiological and clinical features of 'uta' have led some authorities to suggest that the disease is 'oriental sore', due to the importation of *L. tropica* to the New World during post-Columbian times. The developmental pattern of *L. peruviana* in experimentally infected sandflies¹¹ more closely resembles that of *L. braziliensis* rather than *L. tropica*, however, and there is no doubt in our minds that 'uta' is indigenous to the Americas.

Leishmania chagasi (American visceral leishmaniasis)

Human visceral leishmaniasis due to *L. chagasi* (Fig. 6) is almost always fatal unless treated, and as many as 3,621 cases were registered in Brazil alone, up to 1976^{29,30}. Although clearly of much greater importance than cutaneous or mucocutaneous leishmaniasis, the disease is,

fortunately, easy to control because of its domestic or peri-domestic nature: like 'uta', American visceral leishmaniasis is unassociated with forested regions. The major reservoir of infection for man is the domestic dog³¹, although the fact that dogs (and more rarely foxes) generally die from the infection does suggest that some hitherto undetected wild animal reservoir may exist.

The principal vector of *L. chagasi* is almost certainly the sandfly *Lutzomyia longipalpis*, for the distribution of this insect coincides remarkably well with that of the human disease, throughout Latin-America^{11,31}, and it has been shown readily to transmit the parasite, experimentally, to hamsters³². *Lu. longipalpis* is found abundantly in houses and farm-buildings, where it feeds on a wide range of animals, from chicken to man. Strangely enough, it is not markedly anthrophilic and is much more attracted to the dog: American visceral leishmaniasis therefore principally affects these animals; although explosive outbreaks of the human infection occur sporadically, when the number of *Lu. longipalpis* builds up to a high level in the presence of infected dogs.

Although the principal foci of visceral leishmaniasis due to *L. chagasi* are in the drier, poorly forested areas of Latin-America, a small number of human and canine infections have been recorded in the densely forested Amazon region of Brazil^{33,34}. Unfortunately, isolates of the causative parasite are no longer available for study and it is impossible to say if the disease was due to *L. chagasi* or some other leishmania. Although sandflies associated with patients' houses were described as *Lu. longipalpis*, the entomologist Mangabeira later doubted their correct identification³⁵. The very different ecology of Amazonian visceral leishmaniasis and the disease due to *L. chagasi*, elsewhere in Brazil and other Latin-American countries, does suggest that American visceral leishmaniasis may not all be due to the same parasite. Comparative studies need to be made on many isolates from man, dogs, foxes or other wild animals throughout the Americas, and a taxonomic revision made of the sandflies referred to as '*Lu. longipalpis*' from the same regions.

Control and the future

Because of its domiciliary nature, 'uta' (*L. peruviana*) remains the only form of American cutaneous leishmaniasis which has been efficiently controlled by the use of insecticides²⁶. This was fortuitously achieved during intensive DDT spraying campaigns against *Lu. verrucarum*, which had been indicated as the vector of human bartonellosis (Carrión's disease) in the Peruvian Andes³⁷.

Control of American visceral leishmaniasis (*L. chagasi*) has also been effective, for the same reason, in a number of major endemic regions, in particular north-east Brazil^{31,38,39}. It has been based on a three-pronged attack involving the mass extermination of infected and stray dogs, insecticide spraying of houses and farm buildings, and the treatment of infected persons.

The problem of controlling cutaneous and mucocutaneous leishmaniasis in the vast, forested areas of Latin-America at present seems insoluble. The large-scale use of insecticides in tropical rain-forest is not only highly uneconomical, but possibly dangerous from a biological standpoint; destruction of the small-mammal population (or sloths!) would be equally out of the question.

The clearance of forest, in the continuing development of land for agricultural and other purposes, will undoubtedly reduce areas of endemic leishmaniasis. In this respect, it should be realised that one form of leishmaniasis may be eliminated at the risk of encouraging another. Thus, the vectors of Amazonian foci of leishmaniasis due to *L. b. braziliensis* and *L. b. guyanensis* occur principally in the tall 'terra firme' forests. Destruction and subsequent regrowth

of this creates a low, dense 'capoeira' which is highly favourable to *Lu. flaviscutellata* and may, therefore, increase the incidence of cutaneous and diffuse cutaneous leishmaniasis due to *L. m. amazonensis*. Alternatively, pushing back the forest and establishing an open, dry terrain may create new areas which are favourable for *Lu. longipalpis* and accompanying visceral leishmaniasis.

The ideal solution to control of leishmaniasis, particularly in sylvatic situations as discussed above, would clearly be the production of a simple but effective vaccine. The lack of cross-immunity between the various parasites causing American leishmaniasis raises problems in this respect, and it would seem best to think in terms of a polyvalent vaccine. Until this is achieved there remains much room for improvements in treatment of the diseases caused by the different leishmaniae, at present largely limited to prolonged courses of injections with costly and relatively toxic anti-monoclonal drugs. Chemoprophylaxis is another line of research which seems to have been sadly neglected.

From an apparently simple beginning, the study of leishmaniasis has assumed a surprising complexity, particularly in the New World: in spite of the steadily growing list of species and subspecies of *Leishmania*, however, it remains quite obvious that we have but scraped the surface regarding knowledge of the epidemiology, ecology and taxonomy of this fascinating complex of protozoal parasites.

1. Lindenberg, A. *Rev. med. Sao Paulo* **12**, 116-120 (1909).
2. Carini, A. & Paranhos, U. *Rev. med. Sao Paulo* **12**, 111-116 (1909).
3. Vianna, G. *Brasil-Méd.* **25**, 411 (1911).
4. Migone, L. E. *Bull. Soc. Path. Exot.* **6**, 118-120 (1913).
5. Deane, L. M., Deane, M. P. & Alencar, J. E. *Rev. Bras. Malariol. Doenças trop.* **7**, 131-141 (1955).
6. Cunha, A. M. & Chagas, E. *Hospital (Rio de Janeiro)* **11**, 3-9 (1937).

7. Lainson, R. & Shaw, J. J. *Br. med. Bull.* **28**, 44-48 (1972).
8. Lainson, R. & Shaw, J. J. *Bull. Panam. Hlth Org.* **7**, 1-19 (1973).
9. Chance, M. L., Peters, W. & Shchory, L. *Ann. trop. Med. Parasit.* **68**, 307-325 (1974).
10. Gardener, P. J., Chance, M. L. & Peters, W. *Ann. trop. Med. Parasit.* **68**, 317-325 (1974).
11. Lainson, R. & Shaw, J. J. in *The Kinetoplastida 2* (eds Lumsden, W. H. R. & Evan, D. A.) (Academic, London, in the press).
12. Lainson, R. & Shaw, J. J. *J. trop. Med. Hyg.* **80**, 29-35 (1977).
13. Lainson, R. & Shaw, J. J. *Trans. R. Soc. trop. Med. Hyg.* **62**, 385-395 (1968).
14. Lainson, R., Shaw, J. J., Ward, R. D. & Fraiha, H. *Trans. R. Soc. trop. Med. Hyg.* **67**, 184-196 (1973).
15. Lainson, R., Ward, R. D. & Shaw, J. J. *Trans. R. Soc. trop. Med. Hyg.* **70**, 171-172 (1976).
16. Ward, R. D. & Fraiha, H. *J. med. ent.* **14**, 313-317 (1978).
17. Biagi, F. F., Biagi, A. M. & Beltran, H. F. *Prems. Med. Mex.* **30**, 267-272 (1965).
18. Disney, R. H. L. *J. appl. Ecol.* **5**, 1-59 (1968).
19. Fraiha, H., Ward, R. D., Shaw, J. J. & Lainson, R. *Bol. Of. San. Panam.* (in the press).
20. Shaw, J. J. & Lainson, R. *Trans. R. Soc. trop. Med. Hyg.* **62**, 396-405 (1968).
21. Herrero, A., Telford, S. R. & Christensen, H. A. *Ann. trop. Med. Parasit.* **65**, 349-358 (1971).
22. Tikasingh, E. S. *Bull. Panam. Hlth Org.* **8**, 232-242 (1974).
23. Lainson, R., Ward, R. D. & Shaw, J. J. *Proc. R. Soc. B* **199**, 309-320 (1977).
24. Herrero, A., Christensen, H. A. & Beumer, R. J. *Am. J. trop. Med. Hyg.* **22**, 585-591 (1973).
25. Johnson, P. T., McConnell, E. & Hertig, M. *Expl Parasit.* **14**, 107-122 (1963).
26. Tesh, R. B., Chaniotis, B. N. & Johnson, K. M. *Am. J. Epidemiol.* **95**, 88-93 (1972).
27. Herrero, A. *Rev. Med. Exp., Lima* **8**, 87-117 (1951).
28. Herrero, A. *Rev. Med. Exp., Lima* **8**, 119-137 (1951).
29. Deane, L. M. & Deane, M. P. *Arq. Hig. Saúde Publ., Sao Paulo* **29**, 89-94 (1964).
30. Ward, R. D. *Proc. 15th Int. Congr. Ent., Washington, DC* 505-522 (1977).
31. Deane, L. M. in *Leishmaniose Visceral no Brasil* (Serviço Nacional de Educacao Sanitária, Rio de Janeiro, Brasil, 1956).
32. Lainson, R., Ward, R. D. & Shaw, J. J. *Nature* **266**, 628-630 (1977).
33. Chagas, E. *et al. Mems Inst. Osw. Cruz* **33**, 89-229 (1938).
34. Costa, O. R. *Rev. Serv. Esp. Saúde Publ., Rio de Janeiro* **12**, 91-98 (1966).
35. Mangabeira, O. *Rev. Bras. Malariol. Doenças trop.* **21**, 3-26 (1969).
36. Herrero, A. *Rev. Med. Exp., Lima* **10**, 139-145 (1956).
37. Hertig, M. & Fairchild, G. B. *Am. J. trop. Med.* **2**, 207-230 (1948).
38. Alencar, J. E. *Rev. Inst. Med. trop., Sao Paulo* **3**, 175-180 (1961).
39. Sherlock, I. A. & Almeida, S. P. *Rev. Bras. Malariol. Doenças trop.* **22**, 175-181 (1970).

Identification of economically important parasites

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The taxonomy of a parasite can be an important guide to its pathogenic characteristics. A wide range of anatomical, biochemical and behavioural tests is now being developed to define different strains and subspecies of the main tropical parasites.

WHEN a parasite's behaviour is obnoxious to man or his domestic stock, there is a fundamental need to identify the organism, not for slotting into a taxonomic niche, but to help the clinician predict the outcome of the infection and decide how to effect a cure¹. In addition, because of our incomplete knowledge of the occurrence, dissemination and control of many parasitic diseases, it is just as important that the epidemiologist be told about their behavioural characteristics, such as the range of definitive hosts, the host response, tissue tropism, periodicity, survival in a free-living phase, vector-preference, and duration of vector cycle. Some of these factors may vary between parasites that have similar life cycles; for example *Brugia* spp² have different periodicities.

Unfortunately behaviour is often difficult to determine experimentally because of time, expense and the variability, or lack of availability, of host test systems. Thus we require easily recognised intrinsic³ features which are associated with behavioural characteristics and which can easily be examined in many samples of a parasite. Pragmatically, of

course, behavioural and intrinsic characterisation often progress together.

Many qualitative morphological features used for identification reflect the importance of a particular phase in the life cycle, as for instance, the gametocytes of a malaria parasite and the bloodstream microfilariae of a tissue nematode. Thus, with a knowledge of life cycles, we can construct a taxonomy which enables most newly discovered parasites to be confidently identified and assigned to a major taxon on morphological criteria alone, without recourse to experiment. We can be even more confident when morphological characterisation is undertaken at each phase of the life cycle; for example, the recently found intracellular gut phase of *Toxoplasma* allied it to the coccidia⁴, and caused a reevaluation of its epidemiology. Morphological identification, then, can be extremely valuable to the epidemiologist because of what it may tell him about the life cycle of a parasite.

It is in distinguishing specific and subspecific differences in behaviour that morphology can fail. The trypanosomes illustrate this point well⁵, because in this case the successful morphological separation of behaviourally different subgenera led to the definition of species and subspecies solely on minor morphological criteria, with scant regard to

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behavioural characteristics. Sometimes mensural data were not statistically tested, but then if organisms are measured in adequate numbers the advantage that qualitative morphological criteria has for swift identification with a few organisms is lost. Moreover, the apparently successful application of measurement to differentiation may be invalidated as more material becomes available. Again trypanosomes provide a good example: after some years of confusion in the subgenus *Nannomonas*, the two closely allied species, *Trypanosoma congolense* and *T. dimorphon* were separated on mean length measurements, while a third, *T. simiae*, differed from these in a greater length and a unique virulence to pigs⁶; each has a similar cycle and qualitative morphology in the tsetse fly. Later, measurements on a new isolation bridged the gap between *T. congolense* and *T. dimorphon*, bringing these together as *T. congolense*⁷. Then, a form of *T. simiae*, highly virulent to pigs, came into the *T. congolense* range of measurements⁸, once again leaving the subgenus in a confused state. One wonders how many other taxonomic groupings would fall into disarray after more intensive studies.

Newer methods of identification

Behavioural identification must continue to play a fundamental part, but it can be improved by standardisation; whenever valid, morphological identification will remain convenient for intrinsic characterisation, greater accuracy perhaps being gained with the less convenient ultrastructural techniques.

Biochemical identification offers accuracy with reliability and wide applicability. The sequence of bases in DNA would give an exact definition of an organism and several useful indirect approaches are evolving⁹. The ratio of the pairs of complementary bases is reflected in a consistent DNA buoyant density¹⁰. The genetic relatedness of two parasites has been measured by examining the hybrids formed after allowing thermally denatured DNA from each to renature together^{9,11}. Hybridisation of DNA and RNA is also being assessed for identification^{9,12}; RNA made complementary to the DNA of one organism is tested against the DNA of another, the degree of binding being a measure of relationship while only a few organisms need be tested. Restriction endonuclease analysis is another approach to a more refined examination of DNA. Each endonuclease splits DNA at a specific point, the electrophoretic mobilities of each set of cleavage products being the same in identical organisms¹³.

Proteins, the products of the genes, can also be used for identification. At present, the determination of a complete molecular structure is laborious, but part is examined indirectly and conveniently by serological methods, sometimes on only a few organisms, or by cross-immunity tests. A protein's specialised function, for which its structure has evolved, can also be examined, and the generally high specificity of enzymes towards their substrates makes them eminently suitable subjects. Several molecular forms with an identical catalytic function may exist, either in the same or different organisms, and are termed isoenzymes, and their molecular basis is well described^{9,14}. If the molecular differences are associated with differences in charge, the different positions after electrophoresis can be located visually by a colour change linked with the specific, but common, enzyme activity, even in a crude mixture of cell proteins containing other enzymes. Moreover, an enzyme molecule's ability to process many thousands of substrate molecules permits minute amounts of activity to be detected.

Each of the many enzymes in a cell could be a marker for characterisation, thus allowing the possibility of a considerable extension of the range of identifying characters. The more enzymes found with common electrophoretic mobilities, the greater the confidence in a close relationship

between organisms, and this can be as important for identification as those isoenzymes which show clear electrophoretic differences. The true nature of multiple banding of one enzyme in a single sample, which may arise from a mixture of populations or from several isoenzymes in one cell, will be revealed by cloning, bearing in mind the possibilities of artefactual banding. A disadvantage of isoenzyme identification is the large number of cells required, although it has the advantages of simplicity, speed and the possibility of comparing many samples. Improvements may occur with isoelectric focusing, whereby each isoenzyme bands sharply at its isoelectric point on electrophoresis in a pH gradient.

The aim should be to associate a number of biochemical characters with important features of behaviour. An association with a single character can be regarded confidently provided it is consistent in most isolations of the particular organism, but some discrepancy can be expected¹⁵ since a behavioural property may be determined independently of any single intrinsic character; for example, no change occurred in two characteristic isoenzymes of a mild malaria parasite when it suddenly became virulent¹⁷, and drug resistance segregated independently of an isoenzyme marker during genetic recombination experiments with malaria¹⁸.

Examples will be given of a few problems of identification among those economically important parasitic diseases, and their related laboratory models, where the newer methods are being applied, usually for taxonomic purposes. Confusion reigns in the infraspecific nomenclature but the meaning of such terms as strain, stock and variety should be obvious in the context of the discussion on each organism.

Amoebiasis

A major question with *Entamoeba histolytica* is whether two types occur, one which causes the acute symptoms of amoebic dysentery involving tissue invasion, and one which is apparently benign. Isolations from patients with clinical disease are more invasive in rats¹⁹. DNA buoyant density measurements on *E. histolytica* showed differences between strains although the method was believed too insensitive for characterisation²⁰. A difference in surface character between the two putative kinds of *E. histolytica* is indicated by the ability of low concentrations of concanavalin A to agglutinate the pathogens but not the nonpathogens²¹.

The earliest attempts at isoenzyme characterisation of parasitic protozoa were on amoebae which, using five enzymes, could be grouped into two, one which consisted of those which grew at high temperatures *in vitro*, and the other of those which grew at lower temperatures²². The authors suggest that the isoenzyme patterns of the 'low-temperature' amoebae may ally them to *E. moshkowskii*, found in sewage. Comparisons were limited in this early work, but recently more extensive comparisons distinguished the nonpathogens *E. coli* and *Iodamoeba buetschlii* from several groups of *E. histolytica*^{23,24}. More important, one of these groups of *E. histolytica* had a characteristic phosphoglucose mutase isoenzyme which was seen only in those stocks isolated from patients suffering with clinical amoebiasis²⁵. Perhaps future work will establish whether the reports¹⁹ of experimental change in virulence concern selection from a mixed population or the induction of some kind of genetic alteration.

Leishmaniasis

The leishmanias can be roughly divided into four morphologically similar main groups, including the 'visceral' and 'cutaneous' types that infect man; geographical provenance, followed by behavioural characterisation in culture, certain definitive hosts and the vector, is the recommended approach for identification, together with immunological

tests²⁶. Much current work is devoted to improving characterisation, and has included intensive studies on behaviour to define the 'mexicana' and 'braziliensis' complexes in Latin America, now supported by mensural²⁸ and cross-protection criteria²⁹. Differentiation has also been possible with ultrastructural measurements and the numbers of microtubules³⁰.

There is considerable variation in DNA buoyant density within the genus, with clear differences between the New and Old World isolations. Many subgroupings appear, including a distinction in kinetoplast DNA³¹, between the behaviourally different *Leishmania tropica major* and *L. t. minor*. There is also much heterology as shown by RNA/kinetoplast DNA hybridisation within a single species, *L. aethiopica*³², which suggests that it has little value for identification. With the widely used serological methods, identification seems possible although relationships are complex³³. A simpler technique is based on the excretion *in vitro* of a single factor which precipitates with antibody³⁴, and identifies the geographical origin of a strain.

Enzyme polymorphism in malic dehydrogenase supports differentiation by DNA buoyant densities³⁵. Valuable recent work has investigated enzyme variation in a limited area; many isolations from Kuwait were examined with seven isoenzymes, and this reveals six groups in the area, one of which resembles *L. t. major* and another *L. t. minor*³⁶. Of greater value is the integrated approach where nuclear and kinetoplast DNA buoyant densities, five isoenzymes and the serologically active excreted factor have been used to eliminate a suspected animal reservoir of *L. dionisii*, and to confirm the reservoir of *L. aethiopica*³⁷.

Trypanosomiasis

The difficulty of identifying some species and subspecies of trypanosomes has also attracted much interest in recent years⁷, especially in the subgenus *Trypanozoon* which comprises a morphologically similar but behaviourally heterogeneous collection of organisms, two among which, *T. brucei gambiense* and *T. b. rhodesiense* cause respectively a chronic and an acute disease in man, and differ from an animal counterpart, *T. b. brucei*, by the latter's inability to infect man. It is clearly important to know whether a tsetse fly or a mammal is the reservoir of the human disease. The testing of infectivity to man has been simplified and to some extent standardised in the blood incubation infectivity test^{38,39}; the viability of the trypanosomes after exposure to normal human plasma is tested by inoculation into normal laboratory rats or mice. However, since these animals are difficult to infect with many isolations of *T. b. gambiense*, the test was modified by using a more susceptible host, *Mastomys natalensis*, which was also immunologically depressed⁴⁰.

Some hope of morphological characterisation may lie in the quantitative mitochondrial differences, as seen under the electron microscope, between *T. b. brucei* and *T. b. rhodesiense*⁴¹. Biochemical methods have shown that the buoyant densities of the components of nuclear and kinetoplast DNA are similar among the various members of *Trypanozoon*, although an extra satellite DNA seems to characterise *T. b. gambiense*⁷. RNA-DNA hybridisation was too sensitive for differentiating between the major nosodemes in that differences between stocks of *T. b. brucei* were greater than between this organism and *T. b. rhodesiense*⁴². Promise may be shown by restriction endonuclease analysis which showed differences within the subgenus⁴³.

The glycoprotein structures of individual variant-specific antigens are now being precisely analysed^{42,43}, and conceivably may eventually identify, perhaps through a highly specific antibody, the property of human infectivity among *Trypanozoon*, since this was associated with certain antigenic types of trypanosomes⁴⁴.

Isoenzyme characterisation is proving particularly rewarding. An isoenzyme of alanine aminotransferase characteristic of *T. b. gambiense* isolated from man⁴⁵ was also found in trypanosomes⁴⁶ isolated from Liberian pigs, which thus reinforces the suggestion, based on these trypanosomes' resistance to human plasma, that these animals may be reservoirs of human Gambian trypanosomiasis⁴⁰.

Hitherto unrecognised groupings are also being demonstrated by isoenzymes. *T. b. rhodesiense* isolated from particular areas were characterised by various isoenzymes from other *T. b. rhodesiense*⁴⁶; additionally, different aminotransferase isoenzymes distinguished between two serotypes of *T. b. gambiense*⁴⁵. Whether these enzyme-variants are associated with different modes of behaviour remains unknown.

Many problems of identification occur amongst those salivarian trypanosomes with their various degrees of infectivity and pathogenicity to domestic animals, and the epidemiology of the diseases caused is poorly known. Few DNA buoyant densities are reported⁹, but enzyme polymorphism in the cattle pathogen *T. vivax* was used to demonstrate the stability of such polymorphism in the field^{47,48}.

Biochemical characterisation is also helping to solve the many problems concerning the epidemiology of Chagas' disease caused by *T. cruzi* in Latin America⁷. Restriction endonuclease cleavage clearly differentiates between four strains of *T. cruzi*⁴⁹. Buoyant density differences among nuclear and kinetoplast DNA are supported by isoenzyme differences in distinguishing between *T. dionisii* and *T. vespertilionis* of bats in Europe where Chagas' disease is unknown; both are identical morphologically to *T. cruzi* in the bloodstream phase, although a characteristic form of *T. dionisii* appears during culture *in vitro*⁵⁰. Similarly, European *T. vespertilionis* and the Latin American bat trypanosomes were distinguishable, but the isoenzyme patterns were also markedly heterogeneous among the New World organisms⁵⁰. The significance of these previously undetected groupings in relation to bats as reservoirs of Chagas' disease awaits a fuller description of enzyme polymorphism in *T. cruzi* isolated from man.

Epidemiology trenchant divisions, again hitherto unrecognised, of *T. cruzi* were also discovered by isoenzyme electrophoresis. Two markedly different forms circulated independently in domestic and sylvatic cycles^{51,52}, although the 'sylvatic' kind can invade the domestic situation^{52,53}. Further enzyme polymorphism also occurred in *T. cruzi*⁵³, so that gradually the epidemiological significance of the reported variations in disease and of animal reservoirs should emerge.

Coccidiosis

The minor behavioural, morphological and immunological differences between the intracellular gut parasites of the fowl, *Eimeria acervulina* and *E. mivati*, led to the latter's being considered as *E. acervulina* var. *mivati*, but so far a limited range of enzymes has shown no isoenzyme differences⁵⁴⁻⁵⁶.

The reliable cross-immunity tests have also revealed another variety, *E. acervulina* var. *diminuta* from the Ceylon jungle fowl, which was also more sensitive to drugs⁵⁶. The isoenzyme patterns remained constant on the easy transition of this variety to embryo culture, whereas a change occurred in the more difficult adaptation of the *mivati* variety to embryos, which suggests the selection of a population from an original mixture.

Two infraspecific types of the turkey parasite *E. meleagridis* were indicated, unreliably, by oocyst size and categorically by cross-immunity experiments; isoenzymes of lactate dehydrogenase and glucose phosphate isomerase confirmed the division⁵⁷.

Malaria

The rodent plasmodia are popularly used as laboratory models for research on malaria. Thanks to the parallel progress of isoenzyme characterisation, with up to eight enzymes, alongside geographical, developmental and morphological criteria, a rodent malaria from several parts of Africa could easily be identified, and its probable behaviour predicted. However, its name would depend on when the identification is made, since opinions on nomenclature change so rapidly. Currently four species, *Plasmodium berghei*, *P. yoeli*, *P. vinckei* and *P. chabaudi* are stipulated, three of which exhibit subspeciation⁵⁸⁻⁶¹, but this classification may change when material is examined from areas other than the five African areas already studied.

A natural limitation and consistency of enzyme polymorphism have emerged from the examination of a number of isolations of the same organism from one area, although one isolate of *P. vinckei lentum* was distinctly separated off from other *P. v. lentum*⁵⁹. Other work emphasises the need to standardise extraction techniques and to adopt adequate controls regarding interfering host enzymes; malate dehydrogenase is also undetectable in some strains of one species⁶². DNA buoyant densities can separate three species, and DNA-DNA hybridisation experiments confirm some subspecies as distinct entities⁶³. The importance of identifying strains of the human malaria *P. falciparum*, and the failure of immunological methods to do this are stressed⁶⁴; this parasite is polymorphic for three enzymes in The Gambia and elsewhere^{64,65}, and it may be interesting to know, as in the rodent malarias, whether any differences in modes of behaviour occur in either man or the mosquito.

Schistosomiasis

The intraspecific variation in behaviour among schistosomes has much epidemiological significance, although host factors also exert a profound influence⁶⁶. Laboratory investigations have been on only a few isolations from particular areas, but nevertheless confirm the field observations that an individual strain is preferentially transmitted by certain snails⁶⁷⁻⁶⁹; infectivity, growth rates, maturation times, egg production, and tissue egg-deposition also experimentally demonstrate strain differences⁶⁹.

Intraspecific identification by egg shape and size is used, but has serious limitations⁶⁹ which illustrate the need for unequivocal markers. The isoenzymes of malate dehydrogenase and nonspecific esterase vary according to the sex of *Schistosoma mansoni*, and possibly to individual worms in the same host⁷⁰; later work has shown that strains could not be distinguished by using five enzymes, and changes in the malate dehydrogenase patterns occurred during laboratory passage in mice, possibly by population selection⁷¹. However, the similar *S. bovis* and *S. matthei* of ruminants have been compared by behaviour, egg shape and size and three enzymes⁷², although again there are sex differences between the isoenzymes, and intraspecific variation in *S. bovis*; acid phosphatase and malate dehydrogenase are helpful in differentiation. The same two enzymes are helpful in distinguishing *S. intercalatum* from *S. haematobium* and from *S. matthei*⁷³. Resolution of isoenzymes has been improved by isoelectric focusing⁷⁴. Now that single worms can be examined by this method, rapid progress in strain identification may be expected, and the isoenzymes of phosphoglucose isomerase may also be useful for identification¹⁵.

Conclusion

The identification of parasites has arrived at an exciting new phase, when many new characters are becoming available. These, together with the more familiar ones, should be used to complete a detailed dossier for each organism of interest. It never has been, nor can be, a

detached recording but must attempt to identify the harmful, the beneficial and the indifferent, with all the transitional shades of these attributes, so that we may eventually define the problem of each parasitic disease more accurately in order to effect efficient control.

1. Steel, K. J. in *Microbial Classification* (eds Ainsworth, G. C. & Sneath, P. H. A.) 405-432 (Cambridge University Press, London, 1962).
2. Denham, D. A. & McGreevy P. B. *Adv. Parasitol.* **15**, 243-309 (1977).
3. Lumsden, W. H. R. *Trans. R. Soc. trop. Med. Hyg.* **68**, 14-75 (1974).
4. Hutchinson, W. M., Dunachie, J. F., Work, K. & Siim, J. C. *Trans. R. Soc. trop. Med. Hyg.* **65**, 380-399 (1971).
5. Godfrey, D. G. *Protozoology* **3**, 33-49 (1977).
6. Hoare, C. A. *Parasitology* **49**, 210-231 (1959).
7. Godfrey, D. G. *Ann. trop. Med. Parasit.* **54**, 428-438 (1960).
8. Roberts, C. J. *Ann. trop. Med. Parasit.* **65**, 319-326 (1971).
9. Newton, B. A. in *Biology of the Kinetoplastida* (eds Lumsden, W. H. R. & Evans, D. A.) 405-434 (Academic, London, 1976).
10. Borst, P. & Fairlamb, A. H. in *Biochemistry of Parasites and Host-Parasite Relationships* (ed. van den Bossche, H.) 169-191 (North Holland, Amsterdam, 1976).
11. Chance, M. L. & Warhurst, D. C. *J. Protozool.* **20**, Suppl. 524 (1973).
12. Steinert, M., Assel, S. van, Borst, P. & Newton, B. A. in *Genetic Functions of Mitochondrial DNA* (eds Saccone, H. & Kroon, A. M.) 71-81 (North Holland, Amsterdam, 1976).
13. Brack, C., Bickle, T. A., Yuan, R., Barker, D. C., Foulkes, M. & Newton, B. A. in *Biochemistry of Parasites and Host-parasite Relationships* (ed. van den Bossche, H.) 211-218 (North Holland, Amsterdam, 1976).
14. Harris, H. & Hopkinson, D. A. *Handbook of Enzyme Electrophoresis in Human Genetics* (North-Holland, Amsterdam, 1976).
15. Ross, G. C. *Proc. analyt. Div. chem. Soc.* **14**, 76-79 (1977).
16. Godfrey, D. G. & Kilgour, V. *Trans. R. Soc. trop. Med. Hyg.* **70**, 219-224 (1976).
17. Yoeli, M. & Hargreaves, B. *Ann. trop. Med. Parasit.* **69**, 173-178 (1975).
18. Walliker, D., Carter, R. & Morgan, S. *Parasitology* **66**, 309-320 (1973).
19. Neal, R. A. *Bull. N. Y. Acad. Med.* **47**, 462-468 (1971).
20. Reeves, R. E., Lushbaugh, T. S. & Montalvo, F. E. *J. Parasit.* **57**, 939-944 (1971).
21. Martinez-Palomo, A., Gonzalez-Robles, A. & de la Torre, M. *Nature new Biol.* **245**, 186-187 (1973).
22. Reeves, R. E. & Bischoff, J. M. *J. Parasit.* **54**, 594-600 (1968).
23. Sargeant, P. G. & Williams, J. E. *Trans. R. Soc. trop. Med. Hyg.* **72**, 164-166 (1978).
24. Williams, J. E. & Sargeant, P. G. *Trans. R. Soc. trop. Med. Hyg.* (in the press).
25. Sargeant, P. G., Williams, J. E. & Green, J. D. *Trans. R. Soc. trop. Med. Hyg.* (in the press).
26. Garnham, P. C. C. *Bull. Wild Hlth Org.* **44**, 477-489 (1971).
27. Lainson, R. & Shaw, J. J. *Br. med. Bull.* **28**, 44-48 (1972).
28. Shaw, J. J. & Lainson, R. *J. trop. Med. Hyg.* **79**, 9-13 (1976).
29. Lainson, R. & Shaw, J. J. *J. trop. Med. Hyg.* **80**, 29-34 (1977).
30. Gardner, P. J., Shchory, L. & Chance, M. L. *Ann. trop. Med. Parasit.* **71**, 147-155 (1977).
31. Chance, M. L., Peters, W. & Shchory, L. *Ann. trop. Med. Parasit.* **68**, 307-316 (1974).
32. Chance, M. L. in *Biochemistry of Parasites and Host-Parasite Relationship* (ed. van den Bossche, H.) 229-252 (North-Holland, Amsterdam, 1976).
33. Matossian-Rogers, A., Lumsden, W. H. R. & Dumonde, D. C. *Immunology* **31**, 1-19 (1976).
34. Schnur, L. F. & Zuckerman, A. *Ann. trop. Med. Parasit.* **71**, 273-294 (1977).
35. Gardener, P. J., Chance, M. L. & Peters, W. *Ann. trop. Med. Parasit.* **68**, 317-325 (1974).
36. Al-Taqi, M. & Evans, D. A. *Trans. R. Soc. trop. Med. Hyg.* **72**, 56-65 (1978).
37. Peters, W., Chance, M. L., Mutinga, M. J., Ngoka, J. M. & Schnur, L. F. *Ann. trop. Med. Parasit.* **71**, 501-502 (1977).
38. Rickman, L. R. & Robson, J. *Bull. Wild Hlth Org.* **42**, 911-916 (1970).
39. Hawking, F. *Trans. R. Soc. trop. Med. Hyg.* **67**, 517-527 (1973).
40. Mehlitz, D. *Trans. R. Soc. trop. Med. Hyg.* **71**, 86 (1977).
41. Böhringer, S. & Hecker, H. *J. Protozool.* **21**, 694-698 (1974).
42. Bridgen, P. J. & Cross, G. A. M. *Nature* **263**, 613-614 (1976).
43. Cross, G. A. M. *Ann. Soc. belge méd. trop.* **57**, 389-399 (1977).
44. Meirvenne, N. van, Magnus, E. & Janssens, P. G. *Ann. Soc. belge Méd. trop.* **56**, 55-63 (1976).
45. Godfrey, D. G. & Kilgour, V. *Trans. R. Soc. trop. Med. Hyg.* **70**, 219-224 (1976).
46. Gibson, W. *Trans. R. Soc. trop. Med. Hyg.* **71**, 386 (1977).
47. Kilgour, V., Godfrey, D. G. & Na'isa, B. K. *Ann. trop. Med. Parasit.* **69**, 329-335 (1975).
48. Kilgour, V. & Godfrey, D. G. *Ann. trop. Med. Parasit.* **71**, 387-389 (1977).
49. Mattei, D. M., Goldenberg, S., Morel, C., Azevedo, H. P. & Roitman, I. *FEBS Lett.* **74**, 264-268 (1977).
50. Baker, J. R., Miles, M. A., Godfrey, D. G. & Barrett, T. V. *Am. J. trop. Med. Hyg.* (in the press).
51. Miles, M. A., Toyé, P. J., Oswald, S. C. & Godfrey, D. G. *Trans. R. Soc. trop. Med. Hyg.* **71**, 217-225 (1977).
52. Hoff, R., Barrett, T., Miles, M. A., Godfrey, D. G., Sherlock, I., Teixeira, R. & Mott, K. XIV Congresso da Sociedade Brasileira de Medicina Tropical (1978).
53. Miles, M. A. *et al. Nature* **272**, 819-821 (1978).
54. Long, P. L. *Parasitology* **67**, 143-155 (1973).
55. Rollinson, D. *Trans. R. Soc. trop. Med. Hyg.* **69**, 436-437 (1975).
56. Shirley, M. W., Millard, B. J. & Long, P. L. *Parasitology* **75**, 165-182 (1977).
57. Long, P. L. *Parasitology* **75**, 177-182 (1977).
58. Carter, R. *Trans. R. Soc. trop. Med. Hyg.* **64**, 401-406 (1970).
59. Carter, R. *Parasitology* **66**, 297-307 (1973).
60. Killick-Kendrick, R. *Parasitology* **69**, 225-237 (1974).
61. Carter, R. & Walliker, D. *Ann. trop. Med. Parasit.* **69**, 187-196 (1975).
62. Momen, H., Atkinson, E. M. & Homewood, C. A. *Int. J. Biochem.* **6**, 533-535 (1975).
63. Chance, M. L., Momen, H., Warhurst, D. C. & Peters, W. *Ann. trop. Med. Parasit.* **72**, 13-22 (1978).
64. Carter, R. & Voller, A. *Br. med. J.* **1**, 149-150 (1973).
65. Carter, R. & McGregor, I. A. *Trans. R. Soc. trop. Med. Hyg.* **67**, 830-837 (1973).
66. Webbe, G. J. *Helminthol.* **45**, 327-335 (1971).

67. Webbe, G. & James, C. J. *Helminthol.* **45**, 403-413 (1971).
 63. Wright, C. A. & Knowles, R. J. *Trans. R. Soc. trop. Med. Hyg.* **66**, 108-118 (1972).
 69. Pitchford, R. J. *Bull. Wld Hlth Org.* **32**, 105-120 (1965).
 70. Coles, G. C. *Comp. Biochem. Physiol.* **33**, 549-558 (1970).
 71. Coles, G. C. *Comp. Biochem. Physiol.* **38B**, 35-42 (1971).
 72. Southgate, V. R. & Knowles, R. J. *J. nat. Hist.* **9**, 273-314 (1975).
 73. Wright, C. A., Southgate, V. R. & Knowles, R. J. *Trans. R. Soc. trop. Med. Hyg.* **66**, 28-56 (1972).
 74. Ross, G. C. *Comp. Biochem. Physiol.* **55B**, 343-346 (1976).

Current problems in the control of mosquitoes

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Since the incidence of mosquito-borne disease was drastically reduced by the use of DDT in the 1950s, the problem has again worsened and no single method of eradication seems likely to achieve lasting success. It now seems that the best hope lies in the combination of various methods of chemical control and perhaps genetic control to rid the third world of its most important disease sector.

THE science of medical entomology can be dated from Patrick Manson's discovery 100 years ago that mosquitoes act as vectors of a filarial parasite of man. In the ensuing century, some of the ancient insect-borne scourges have been brought substantially under control. Plague, which as the Black Death once wiped out a quarter of the population of Europe, has been reduced to between 1,000 and 6,000 cases annually in the world, with ~100-200 deaths¹. Typhus, which infected some 30 million people in Eastern Europe after the first World War and killed about 10%, is now largely restricted to parts of Africa, with 10,000 to 20,000 cases a year and, again, a few hundred deaths².

Cases of mosquito-borne disease, however, are of a different order of magnitude; and the outlook for future reduction is gloomy. Malaria is still the outstanding vector-borne disease, with about 2,000 million people living in potentially malarious areas. Extensive use of that somewhat maligned insecticide DDT greatly reduced the extent of active transmission, to affect only 400 million people. But permanent success has only been achieved around the periphery. The areas of highest endemicity, such as Central Africa, were scarcely affected; and moderately endemic countries in Asia and Central America, after a decade of improvement, are now suffering a serious reversal of the trend. Thus, in India, the annual number of cases rose from the all-time low of 40,000 in 1966 to 1.7 million in 1972 and to 5.8 million in 1976³. Similar alarming statistics could be quoted for Pakistan, Sri Lanka and Central American countries. Overall, the total number of people in the world freed from malaria, after rising from 317 million in 1961 to 728 million in 1971, fell to 436 million in 1976⁴.

Malaria is a familiar term, even to those who have never ventured into the tropics, perhaps because it was so often fatal to early explorers and colonisers. Filariasis is less well known, partly because it seldom affects expatriates, but also because it is less malignant, though it can cause the terrible disfigurement and disablement of elephantiasis. Our attempts to control this disease give little cause for satisfaction, since there are probably more cases of it in the world today than when Manson first incriminated mosquitoes as the vectors. One reason for this is the enormous growth of population in the fertile, humid tropics, where there are about 400 million people at risk⁵. Many of them will be infected by the urban vector, *Culex pipiens fatigans*, in the burgeoning slums round so many tropical towns; and there is every prospect that this problem will increase.

The situation regarding mosquito-borne virus diseases is somewhat less depressing. The risk of yellow fever epidemics in tropical cities has been reduced, although the ineradicable

reservoir of jungle yellow fever imposes the need for indefinite vigilance. The appearance of the malignant haemorrhagic form of dengue in South East Asia presents a difficult problem; but the numbers of cases involved in epidemics are of the order of thousands, not millions.

These variations in success in controlling tropical diseases call for explanation. It could be suggested that substantial advances in disease suppression have been made only in diseases which invade the temperate regions (plague, typhus, peripheral malaria), or those which affect expatriates or the wealthier citizens of tropical cities. While this is roughly true, it should not be concluded that it is largely due to the self-interest on the part of those with the scientific and technical resources. The main reason is that these diseases have been easier to overcome in both situations. Thus, in temperate regions, vector-borne diseases are handicapped both by the less abundant insects and by the longer delay in the maturation of the parasite in them. Above all, the higher living standards had eliminated much disease before scientists and physicians made any substantial contribution. On the other hand, modern medical advances have benefited the educated and more affluent citizens in the tropics, who have been able to take advantage of vaccines and prophylactic drugs. An obvious reason for this is that both are expensive; even a relatively cheap vaccine against measles costs nearly as much as the total sum *per capita* available for health measures in many African countries⁶. Moreover, there are other difficulties; for example, for many diseases vaccines are not yet available, including malaria and filariasis, although new vaccines are being developed. Adequately safe antimalarial prophylactic drugs are available; but the problem here is to ensure that they are universally distributed and regularly taken. (Simple rural folk are lax in persevering with prophylactics when they are not obviously ill.) Malaria suppression in an endemic area is likely to prevent those treated from acquiring the premunition which they would otherwise obtain; so that, on cessation of drug-taking (for any reason) they would be very susceptible to the disease. To overcome this problem, drugs have been added to the salt supply, on the same principle that fluoride is added to drinking water to prevent dental caries. This has had only limited success, however, and the latest WHO Malaria Committee advocated drug distribution only to the most susceptible members of the population: children and pregnant women. Moreover, it must be recognised that drug-resistant strains of *Plasmodium* constitute a problem parallel to that of insecticide resistance.

As regards filariasis, there are drugs which will kill both micro- and macrofilariae; but they must be administered under medical supervision, because of dangerous side effects. Research is in progress to find safer drugs, but no one can forecast when they will be available.

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The value of vector control

This rather long preamble should have explained the difficulties of controlling disease in poor, backward, rural areas by a direct attack on the pathogen. Hence the immense value of the new synthetic insecticides introduced after the Second World War, because they provided the opportunity of controlling these diseases simply and cheaply. In the early days of DDT the cost of protecting an individual from malaria was only a few shillings a year. The success of the first decades of these insecticides has been eulogistically assessed by J. W. Wright of the WHO⁷, and L. J. Bruce-Chwatt has pointed out that DDT has saved some 15 million lives by malaria control alone⁸.

Unfortunately, the early achievements of residual insecticides have been gradually eroded by two drawbacks which were largely unforeseen—insecticide resistance and environmental residues. Yet on both counts, the spraying of dwellings with DDT which achieved so much, was almost innocent. The widespread traces of insecticide in the environment are almost entirely due to agricultural operations, which account for 97% of insecticide usage and involve direct spraying on to the land. Also, house-spraying is less likely to provoke resistance, since it selects only a portion of the insect population—the more dangerous members which enter houses to take blood meals. In consequence, resistance was slow to develop among anophelines and is still absent in a considerable number. Furthermore, many cases of resistance among mosquitoes have been caused by the contamination of their breeding grounds by the run-off from crop spraying. In the course of three decades, however, resistance has proved a grave problem in the control of malaria vectors and has postponed indefinitely the ambitious WHO plan for the global eradication of the disease. It has appeared so far in 42 species of anophelines and severely restricts control in areas containing at least 256 million people, or 29% of the total population living in malarious zones⁹.

The culicine mosquitoes, which are the main vectors of filariasis and the virus diseases, have presented an even more difficult problem. For various reasons, their control by house-spraying has been less satisfactory and they have been largely attacked by larvicides in their breeding grounds. This seems to involve more risk of environmental pollution, although the doses used are light compared with those used on a tropical crop such as cotton. Certainly the more extensive selection of larval populations has been very prone to develop resistance. Some of the earliest cases among mosquitoes occurred in those American species sprayed intensively because of the nuisance of their bites. (These are the salt marsh breeders in Eastern USA and floodwater species in California.) The urban culicines also were repeatedly treated, so that resistance soon appeared in *Culex p. fatigans* the filariasis vector and *Aedes aegypti* vector of yellow fever, dengue (and its haemorrhagic form) and the encephalitis.

The interesting problem of resistance has attracted so much research that the published papers would easily fill a volume of an encyclopaedia; but no method has been discovered which will restore the potency of an insecticide against a pest which has fully developed resistance. The only practical solution has been to change to another type of insecticide, with the result that the cheap and effective organochlorine compounds have been abandoned in many campaigns.

The WHO, which has always had a keen interest in the danger of resistance, soon became aware of the threat to vector control and, in 1960, initiated a collaborative programme, involving laboratories in several countries, to examine all available insecticides for alternatives¹⁰. After a decade in which some 1,500 chemicals had been tested, the only suitable ones found were a few organophosphorus and

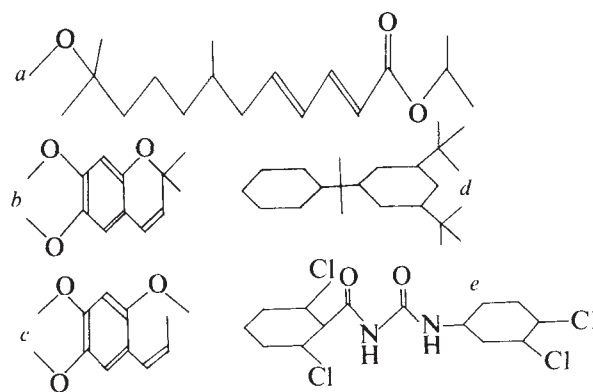


Fig. 1 Formulae of some new pest control agents. a, methoprene; b, precocene II; c, β -asarone; d, MON .0585; e, diflubenzuron.

carbamate compounds. These were at least 10 times as expensive as DDT, a serious matter for impecunious tropical countries. Moreover, these too are beginning to fail, as resistance has been reported in 6 anopheline and 18 culicine mosquitoes, despite less extensive use of insecticides.

The search for new types of insecticide

As resistance gradually reduced the relatively few kinds of insecticides available, those responsible for control programmes waited impatiently for the introduction of new types. But the vigorous campaigns of the environmentalists had stirred various governments into imposing enormously expensive screening procedures to ensure that any new chemicals to be widely used were entirely safe. Now it is necessary to investigate not only acute and chronic toxicity to vertebrates, but possible carcinogenicity, teratogenicity and mutagenicity; furthermore there must be evidence to show that wildlife will not suffer. Thus, the cost of finding, testing and marketing a new pesticide runs to several million pounds. Commercial firms realise that they would be unlikely to recover costs and begin to make a profit without 6 or 7 years of extensive sales; and in the meantime, resistance could restrict usage.

Despite these handicaps, some new types of chemical control materials have appeared in recent years (Fig. 1). They seem to have solved the problem of leaving residues harmful to man and animals by attacking physiological systems in insects which are absent in vertebrates: specifically, moulting and metamorphosis. Best known of these 'third generation' insecticides (or insect development inhibitors) are the hormone mimics. These are commercial developments following a long period of basic research on insect endocrine systems. Practical use of the moulting hormone or its analogues has not been possible, because of their elaborate synthesis and poor penetration into insects. Several firms, however, have been interested in developing juvenile hormone mimics and one (methoprene) has been on the market for several years. These act, apparently, by flooding larvae with hormone mimic at a time when the natural juvenile hormone should decline, and the presence of bogus hormone prevents normal adult development.

An alternative method of interfering with development is to antagonise the synthesis of natural juvenile hormone and thus cause premature metamorphosis. This is apparently the mode of action of precocene I and II, which have recently been described¹¹. Another new compound which interferes with metamorphosis is 'MON 0585', a relatively simple hydrocarbon, which does not seem to be a hormone mimic or an antagonist¹². Its mode of action is obscure and there have been few recent publications on it.

A defect of these compounds which disturb metamorphosis is that they act only at a rather limited period during

juvenile development. Another new type of compound, diflubenzuron, has the advantage of interfering with all moults, apparently by inhibiting chitin synthesis¹³.

All these insect development inhibitors are highly potent against mosquito larvae and have shown promise in field trials. It has therefore been a great disappointment to find that strains of insects which had developed resistance to a series of conventional insecticides have accumulated enough defense mechanisms to show distinct tolerance of these new compounds, even before they have been extensively used in practice. In particular, these multi-resistant strains tend to develop enhanced microsomal oxidase systems, capable of degrading a wide variety of toxicants¹⁴. These could also initiate other kinds of new control agent; for example, extracts of garlic, which seem to be good larvicides¹⁵. There is also a report of a new chemosterilant derived from a plant (β -asarone, from *Acorus calamus*, the sweet flag)¹⁶. This lacks the aziridine ring of the alkylating type of chemosterilant and may perhaps be safe enough to use in practice.

Alternatives to chemical control

Since chemical control measures involve the drawbacks of resistance and residues, a great variety of alternatives has been considered, some old, some new. The long established method of larvicidal oiling has been improved by the discovery of a more potent hydrocarbon mixture known as 'Flit MLO' (ref. 17). Mucilaginous seeds of various cruciferous plants have been used to entangle mosquito larvae¹⁸. Thin films of lethicin spread over water surfaces will prevent mosquito pupae from breaking the surface, so that they eventually drown¹⁹.

Above all, there is hope that 'biological control' may solve the problem. Fifteen years ago, Rachel Carson wrote²⁰: "A truly extraordinary variety of alternatives to chemical control is available. Some are already in use and have achieved brilliant success. Others are in the stage of laboratory testing. Still others are little more than ideas in the minds of imaginative scientists, waiting for the opportunity to put them to the test. All have this in common: they are 'biological' solutions." Biological pest control not only appeals to idealistic environmentalists, but also offers a challenge to ingenious entomologists, pathologists, geneticists and others. Furthermore, the twin pressures of control failure and the environmentalist 'lobby' have facilitated the allocation of research funds to any scheme remotely promising a solution. So the last 20 years have seen a number of novel biological control methods tried out, but with little practical success. The method of control by insect parasites or predators had already been thoroughly explored in the previous 30 years. Its main successes have been in cases where a pest has been brought to a new country without its natural enemies; and there seem to be no striking examples in medical entomology. More recently, attention has been turned to the possibility of control by microorganisms, such as bacteria, protozoa, fungi or viruses. A few such organisms have been tried against mosquito larvae; they include the microsporidians *Nosema* and *Thelohania*, a few fungi and nematodes. However, apart from technical difficulties in culturing and dispersing them, they do not seem to be very reliable control agents. Somewhat more effective are various larvivorous fish, especially *Gambusia affinis*, the mosquito fish. Since, however, this method of control has been known for over 70 years, it is unlikely to provide a complete solution to our present difficulties.

For a more modern and technically demanding approach, genetic control seems to offer a range of fascinating possibilities. These fall into two general types: those that aim at elimination of the pest and those that aim at replacing it with a harmless strain or related species. The attempts at elimination comprise the following methods. (1) The release

of sterilised insects (preferably only males) to mate with the wild females and prevent their reproducing successfully. The sterilisation can be done by exposure to irradiation or by treatment with chemosterilants. (2) The release of males of a closely related species or a different strain of the pest, which will mate with the vector and produce sterile hybrids. Suitable strains may be available in nature, or can perhaps be produced artificially by irradiation, or by radiomimetic chemicals. Both these methods require the production of enormous numbers of insects for release, in order to overwhelm the normal wild males. Alternatively, (3) deleterious genes could be introduced into the wild population by carrier strains equipped with 'meiotic drive' mechanisms to spread these genes. This procedure, though theoretically sound, involves genetic manipulations of considerable complexity.

The idea of species replacement is especially relevant to medical entomology, in that many species would be of negligible importance if they did not transmit disease. Vector capacity has long been known to be under genetic control and recent research is revealing the genes responsible. It is therefore conceivable that a vector species could be replaced by a very similar form with lower or even negligible vector capacity. Possibly the spread of such a benign strain in the field could be facilitated by incorporating resistance factors in the genotype.

This brief outline cannot convey the complex technical problems involved in these different operations, which are well described in the book by Davidson²¹. However, in attempting to assess the relevance of genetic control methods to the reduction of mosquitoes, it must be realised that only methods (1) and (2) have been developed far enough for practical trials. Furthermore, while the subject has been studied for some 30 years, there has only been one large-scale success, the well publicised eradication of the screw-worm fly from Florida. This screw-worm was unique in being unusually sparse in nature, with winter populations falling to only 10 per square mile. To overwhelm them, the release of 800 sterilised flies per square mile was required. Most insect pests, however, and especially mosquitoes, are much more populous with several hundred thousand per square mile. The rearing of such large numbers involves obvious problems, not to mention the necessity of excluding the biting females from those released. Nor is this the only difficulty. The screw-worm campaign aimed at eradicating the flies from the peninsula of Florida, after which the major expenses ceased and were replaced by more modest quarantine measures. But with pests dispersed over large continents, sterilisation measures would have to be maintained permanently.

Despite these intimidating difficulties, there have been several extensive practical trials of genetic control of mosquitoes, both culicine and anopheline. (The largest effort in India, against *Culex p. fatigans*, grounded on the rocks of political dissention²². These trials produced some interesting information on mosquito ecology, but can hardly be said to be practically encouraging for impoverished countries suffering from malaria or filariasis.)

Reluctantly, it must be concluded that nothing new has been found which is remotely equivalent to the synthetic insecticides for controlling mosquito vectors of disease. In reviewing their main defects (resistance, residues and rising costs) we must find a crumb of comfort in the fact that they all point to the same method of alleviation: economy in use. Perhaps this can be achieved without failure to prevent disease transmission by integrating chemical control with prophylaxis and vector control by other means. The old-fashioned method of species sanitation by source reduction should not be forgotten. Mosquito breeding sites are often created or enlarged by human activities. Their harmful effects can generally be reversed; by filling in borrow pits, by planting or cutting down vegetation, by draining or

flooding, according to species. Other methods may be even simpler; there is reason to believe that screening of houses or the use of mosquito nets can substantially reduce infection of filariasis³. What is needed is the combination of a good ecologically trained entomologist with cooperative local labour.

The topic of 'integrated control' is popular in several spheres of applied entomology; but it is not easy to make useful generalisations about it. Each mosquito problem requires thorough investigation to find the appropriate solution. This will demand a greater versatility in applied entomologists than in the days when synthetic insecticides provided the universal answer.

1. *Wld Hlth Org. Chronicle* 27, 369-370 (1973).
2. *Wld Hlth Org. Chronicle* 28, 427-428 (1974).

3. *Time Magazine*, September 12, 56, (1977).
4. *Wld Hlth Org. Chronicle* 32, 9-17 (1978).
5. Nelson, G. S. *Trans. Proc. Symp. Medical Entomology Centenary R. Soc. trop. Med. Hyg.* 15-25 (1977).
6. Hendrickse, R. G. *Trans. R. Soc. trop. Med. Hyg.* 69, 31-34 (1975).
7. Wright, J. W. in *The Future of Insecticides* (eds Metcalf, R. L. & McKelvey J.J.) (Wiley, London, Sydney and Toronto, 1967).
8. Bruce-Chwatt, L. J. *Bull. Wld Hlth Org.* 44, 419-424 (1971).
9. *World Health Organisation 22nd Rpt Expert Committee on Insecticides, Tech. Rpt Ser. No. 585* (1976).
10. Wright, J. W. *Bull. Wld Hlth Org.* 44, 11-12 (1971).
11. Cupp, E. W. *et al. Ent. exp. appl.* 22, 23-28 (1977).
12. Sacher, R. M. *Mosquito News*, 31, 513-516 (1971).
13. Mulder, R. & Gijswijt, M. J. *Pestic. Biochem. Physiol.* (1973).
14. Plapp, F. W. & Vinson, S. B. *Pestic. Biochem. Physiol.* 3, 131-136 (1973).
15. Amonkar, S. V. & Banerji, A. *Science*, 175, 1343-1344 (1971).
16. Saxena, B. P. *et al. Nature*, 270, 512-513 (1977).
17. Micks, D. W. *et al. J. econ. Ent.* 60, 426-429 (1967); 61, 647-650 (1968).
18. Reeves, E. L. & Garcia, C. *Mosquito News*, 29, 601-607 (1969).
19. McMullen, A. I. & Hill, M. N. *Nature*, 267, 244-245 (1977).
20. Carson, Rachel, *Silent Spring*, 304, (Hamilton, London, 1963).
21. Davidson, G. *Genetic Control of Insect Pests* (Academic, London & New York 1975).
22. *Nature* 256, 355-357 (1975).

Principles of the eradication or control of tsetse flies

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The success of attempts to control African trypanosomiasis afflicting both men and animals through the destruction of the tsetse vector depends on a realistic assessment of human and ecological factors in infested regions. The complete eradication of tsetse is at present possible only in limited areas, and elsewhere the advantages of periodic control campaigns have to be weighed carefully against their cost.

ECONOMICALLY crippling diseases of man and his domestic livestock in Africa are caused by pathogenic trypanosomes (*Trypanosoma* spp.) transmitted by tsetse flies (*Glossina* spp.). These diseases can be controlled by the use of trypanocidal drugs (both curative and prophylactic), by the re-settlement of human or animal populations at risk, by the exploitation of trypano-tolerant livestock and by vector control. At present the only way to eliminate the disease is to eradicate the vector. This method, however, is practicable only in special circumstances. An attempt is made here to outline some principles on which anti-tsetse operations should be conducted.

Techniques for tsetse eradication and control

Almost all methods used at present in anti-tsetse operations depend on insecticides. The clearing of vegetation, on which tsetse depend for shelter, and the destruction of game animals, on which they depend for food, were widely used in the past; and other techniques based on biological and genetical approaches may become available in the future. A comprehensive review of the methods available is given by Mulligan¹; more recent reviews^{2,3} have concentrated on current insecticidal techniques and possible future developments.

Of the various insecticidal methods, only the application of residual formulations of insecticide to known tsetse habitats, either from the ground⁴⁻⁶ or by helicopter⁷, has proven efficacy. The sequential application of very low volumes of non-residual formulations of insecticide from the air^{8,9} can give a high degree of control of tsetse populations but cannot yet be said to have achieved eradication.

Tsetse eradication

For technical and economic reasons there are major advantages in eradicating *Glossina* from a prescribed area and then protecting the area from subsequent reinvasion, either by creating barriers or by modifying the environment by settlement and/or development to such an extent that tsetse can no longer survive. However, this is only technically feasible at present in a relatively small proportion of the area of Africa infested by tsetse flies. Before an eradication campaign can be initiated, two fundamental technical questions have to be answered. First, is a technique available which is known to be capable of eradicating *Glossina* in local conditions and, second, are there enough trained staff to undertake the necessary detailed pre-eradication surveys, the eradication operations themselves and post-eradication surveillance? It is also essential to take into account the species of tsetse present, any natural features limiting their distribution, climatic, vegetational and edaphic factors and the potential of infested land for development, which vary fundamentally from area to area and will dictate the optimum eradication strategy.

It is now widely accepted that the tsetse and animal trypanosomiasis problem should be seen as a problem of land use^{10,11}. With acceptance of this principle has come an increasing realisation of the economic implications of tsetse and trypanosomiasis eradication campaigns. The costs of eradicating *Glossina* from an area (which is a once-only operation, unlike control operations which are periodically repeated) may be relatively insignificant in the light of the economic advantages gained. For example, the infested area may be an obstacle to the development of neighbouring tsetse-free areas; or there may be a risk of migration of tsetse into existing tsetse-free areas; or, of course, the infested area itself may contain resources desirable for immediate economic development¹¹.

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Not all eradication campaigns in the past have complied with these technical and economic criteria. In particular, too little attention has sometimes been given to the rationale of tsetse eradication in terms of local demand for the land and intended land use patterns after removal of tsetse.

A well planned, and certainly the most extensive, tsetse eradication campaign began in 1955 in Nigeria and is still continuing^{5,12-14}. Before operations began, trials were conducted on insecticide application methods, equipment, dose rates and so on, concurrently with studies on the ecology of the *Glossina* species concerned, with the objective of developing methods for the most economical, and thus least contaminating, use of insecticide. The development of methods which were both discriminative (spraying only certain types of vegetation within the infested zone) and selective (spraying only certain parts of trees and shrubs) was of prime importance. By following these principles, in drier areas at the northern limit of tsetse infestation, spraying was limited to small parts of the overall habitat; and even in the moister Northern Guinea Savannah vegetation zone further south, only 10% of the total infested area had to be sprayed.

The campaign began in the far north, along the river systems draining into Lake Chad, but before completion of the operation, detailed studies were undertaken of the feasibility of eradicating tsetse from two further extensive areas, in north-east and north-central Nigeria. Detailed reports (P. E. Glover and P. J. Aitcheson, unpublished) were submitted to the Nigerian authorities and many of the recommendations have been implemented in the subsequent tsetse eradication campaigns. By the end of the 1975-76 spraying season some 194,500 km² had been reclaimed, mainly by the application of residual insecticide from the ground, but including some 9,800 km² cleared by the application of residual insecticide from helicopters. Plans have been made to complete the clearance of tsetse from almost 259,000 km² by 1986.

Complementary to the two feasibility studies, land resource studies were undertaken in north-east^{15,16} and north-central Nigeria (Land Resources Division, Directorate of Overseas Surveys, unpublished). In the north-east study, 10 categories of land use potential were identified and recommendations were made for the optimum utilisation of land cleared of *Glossina*. The same study emphasised that land infested with savannah species of *Glossina* is in effect a virgin resource, affording a unique opportunity for planned development; and that the preparation of enforceable working plans and of legislation to reserve appropriate areas for pastoral and other activities should be initiated before starting anti-tsetse operations.

The Nigerian tsetse eradication operations have been treated in some detail because they provide a good example of the detailed technical and land use planning desirable in such operations. Limited space precludes consideration of smaller eradication campaigns conducted elsewhere and it is necessary to consider now the alternative of tsetse control.

Tsetse control

Despite the technical and economic advantages of eradication operations, vector control is the only practical anti-tsetse approach in much of tropical Africa. Tsetse control involves the killing of enough flies to allow man and his domestic animals to live in an area with limited risk of trypanosomiasis. Controlled areas are generally not isolated from nearby infested areas and control measures therefore have to be repeated regularly which, while often technically simple, may become expensive. Clearly a careful assessment should be undertaken, taking into account technical, economic and social factors, before making any commitment to possibly long-term tsetse control operations. Over vast areas it should be accepted that control is not at present justifiable.

Cost should be a relatively minor consideration in the decision whether or not to use vector control as a means of reducing the incidence of sleeping sickness. The former Nigerian Sleeping

Sickness Service cleared the vegetation bordering some 10,600 km of rivers and streams between 1938 and 1958 (ref. 17), removing the habitat of those species of *Glossina* responsible for the transmission of human trypanosomiasis. More recently, tsetse populations in foci of the disease in Nigeria have been controlled by the use of insecticides; many such areas are now free of disease because they have been included in the extensive eradication campaigns, designed primarily to eliminate animal trypanosomiasis, which I have discussed above. Tsetse control was also effective in controlling outbreaks of human trypanosomiasis between 1950 and 1973 in Western Kenya¹⁸, where there is a continual threat of re-invasion of cleared areas from infested areas in neighbouring Uganda and Tanzania.

Panzootics of rinderpest swept over much of Africa in the last decade of the nineteenth century, decimating the game and coincidentally tsetse populations¹⁹. Many of the control operations conducted over the years, particularly in eastern and southern Africa, represent costly attempts by the authorities to prevent savannah species of *Glossina* from becoming re-established in what is frequently habitat to which they are well adjusted and where they flourished before the panzootic. A commitment to such control, just to maintain the *status quo* and not to release more land for development, requires careful evaluation in both economic and social terms. Operations in progress in the Okavango Delta, Botswana afford an example of tsetse control being undertaken to prevent an expansion of an existing infestation of *G. morsitans*, which would have a substantial effect on both human and animal health and on current land use practices. Tsetse control was formerly effected by vegetation clearing and game destruction but is now effected by insecticides applied from the ground and, particularly, by sequential ultra-low-volume applications of insecticide from the air^{20,21}. Large areas of land have been reclaimed but regular treatment is required to control reinfestation over a long vulnerable perimeter. In countries where a control capability does not exist, advances of savannah tsetse can continue unchecked—such a situation probably exists in southern Angola today.

It is possible to protect small enclaves, such as ranches, stock farms and settlement areas, within major fly belts, by vegetation clearing or by regular applications of insecticide. Such operations are invariably uneconomic, particularly with current escalating costs of labour and materials. For this reason past schemes of this type in a number of countries have, in the main, been discontinued.

Conclusions

Techniques for the eradication of *Glossina* spp. from the drier savannah regions of Africa exist although sufficient trained personnel to undertake large-scale anti-tsetse campaigns do not exist in most African countries. However, assuming that this deficiency can eventually be remedied, and with the increasing impetus of a campaign to control trypanosomiasis over 7 million km² of Africa²², decisions will have to be taken on whether, and if so where, to proceed with extensive tsetse eradication and control campaigns. Some²³ would argue that extensive campaigns should not be undertaken for fear of dire ecological consequences, including overgrazing of cleared areas. Clearly, such views must be carefully examined but the evidence from the Nigerian eradication campaigns so far is that in the short term, far from increasing overgrazing, the removal of tsetse has released extensive new areas for cattle grazing and reduced pressure on the arid naturally fly-free areas further north. The indications from Nigeria are that in the long term the main danger to be guarded against in tsetse eradication campaigns, especially in areas of rapidly increasing human population, is not overgrazing but haphazard land development including the encroachment of subsistence farming into areas of pasture.

The strongest possible case should be made for the close integration of tsetse eradication proposals with planned land development, taking due cognisance of traditional agricultural

and livestock practices. Additionally, the administrative political authority must exist to enforce agreed land usage proposals. If these criteria can be satisfied, the selection of suitable areas becomes a technical problem. There is no point in selecting an eradication area in the middle of the tsetse infested area of Africa. Rather fly populations at the edge of the infested area should be chosen, where they are already under some stress imposed by climatic factors; discrete belts of infestation are particularly appropriate areas for eradication. Detailed studies, based on aerial and painstaking ground surveys to determine the limits of infestation are an essential prerequisite.

Tsetse control campaigns also require the same detailed approach. However, in this case, special attention must be paid to the expected costs of continuing operations relative to expected benefits. Benefits can be substantial—as when a large fly-free area is protected from invasion by tsetse by a relatively minor operation—or benefits can be far less, in economic terms, than the costs of continual control operations.

It has been suggested, particularly in the news media, that the use of toxic chemicals in anti-tsetse operations is a major environmental hazard. Considerations of space preclude more than brief reference here, but studies^{24–28} indicate, in general, that insecticide applications in anti-tsetse campaigns cause no major permanent adverse effects on the environment, particularly non-target organisms. *Glossina* spp. are highly susceptible to the insecticides used and very small quantities, applied in a highly selective manner, and usually once only, are used in such campaigns. As the major objective of tsetse eradication is generally to alter land usage, such change will result in far greater environmental modification than any effect of the chemicals used to achieve eradication²⁹. The use of chemicals in major anti-tsetse operations must be closely monitored but emotive arguments concerning the direct effects of anti-tsetse operations should not be allowed to divert attention from the fundamental implications of removing tsetse flies and consequent changing patterns of land use.

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1. Mulligan, H. W. (ed.) *The African Trypanosomiasis* 950 (Allen and Unwin London, 1970).
2. Hamon, J., Baldry, D. A. T., Parker, J. D., Chailier, A. & Stiles, A. R. in *Tsetse: the Future for Biological Methods in Integrated Control* (ed. Laird, M.) 35–43 (IDRC, Ottawa, 1977).
3. Jordan, A. M. in *Medical Entomology Centenary: Symposium Proceeding*, *Trans. R. Soc. trop. Med. Hyg.* 76–84 (1978).
4. Wilson, S. G. *Bull. ent. Res.* 44, 711–728 (1953).
5. Davies, H. J. *appl. Ecol.* 1, 387–403 (1964).
6. Wooff, W. R. *Int. Sci. Comm. Tryp. Res. Tenth Mtg Kampala* 157–166 (Commission for Technical Cooperation in Africa, Lagos, 1965).
7. Spielberger, U., Na'isa, B. K. & Abdurrahim, U. *Bull. ent. Res.* 67, 589–598 (1977).
8. Lee, C. W. *Bull. Wild Hlth Org.* 41, 261–268 (1969).
9. Lee, C. *Agric. Aviat.* 18, 6–17 (1977).
10. MacLennan, K. J. R. in *The African Trypanosomiasis* (ed. Mulligan, H. W.) 799–821 (Allen and Unwin, London, 1970).
11. Jahnke, H. E. *The Economics of Controlling Tsetse Flies and Cattle Trypanosomiasis in Africa Examined for the Case of Uganda* (Weltforum Munich, 1974).
12. Davies, H. J. *appl. Ecol.* 8, 563–578 (1971).
13. MacLennan, K. J. R. & Na'isa, B. K. *Int. Sci. Coun. Tryp. Res. Thirteenth Mtg. Lagos* 303–309 (Organisation of African Unity, Lagos, 1972).
14. Nigeria, Federal Republic. *Federal Livestock Department, Tsetse & Trypanosomiasis Division, Annual Report, 1973–75* (Rufai, Kaduna, 1976).
15. *The Land Resources of North East Nigeria*. Land resources study No. 9 (Land Resources Division/Directorate of Overseas Surveys, 1972).
16. de Leeuw, P. N. *Savanna* 5, 61–74 (1976).
17. Steiner, J. O. *Tsetse Control, in Rural Health Report, 1963, Min. of Health, Northern Nigeria* (Govt Printer, Kaduna, 1964).
18. Maina, A. *Int. Sci. Coun. Tryp. Res. Cont. Fifteenth Mtg. Banjul* (Organisation of African Unity, Lagos, in the press).
19. Ford, J. *The Role of the Trypanosomiasis in African Ecology. A Study of the Tsetse Fly Problem* 568 (Clarendon, Oxford, 1971).
20. Kendrick, J. A. & Alsoj, N. *PANS* 20, 392–399 (1974).
21. Lee, C. W., Pope, G. G. & Bowles, J. *Centre for Overseas Pest Research Misc. Rept No. 29* (1977).
22. F.A.O. *Programme for the Control of African Animal Trypanosomiasis* FAO/AG/TRYP/74/1E, 18 (1974).
23. Ormerod, W. E. *Science* 191, 815–821 (1976).
24. Koeman, J. H. *et al. Three years Observation on the Side-Effects of Helicopter Applications of Insecticides Used to Exterminate Glossina Species in Nigeria* 51 (Univ. Wageningen, 1976).
25. Koeman, J. H., Le Roux, J. G. & Pennings, J. H. *An Orientation Survey on the Side-Effects and Environmental Distribution of Dieldrin in a Tsetse-Control Area in S.W. Kenya* 35 (Univ. Wageningen, 1969).
26. Koeman, J. H., Rijkse, H. D., Smies, M., Na'isa, B. K. & MacLennan, K. J. R. *Netherlands J. Zool.* 21, 443–463 (1971).
27. Chapman, N. G. *Trans. Rhod. Sci. Ass.* 57, 12–21 (1976).
28. Langridge, W. P. & Mugutu, S. P. *Int. Sci. Coun. Tryp. Res. Twelfth Mtg. Bangui* 195–201 (Organisation of African Unity, Lagos, 1972).
29. MacLennan, K. J. R. *Trop. Anim. Hlth Prod.* 5, 40–45 (1973).

The pathology, pathobiology and pathogenesis of schistosomiasis

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Although the general pathology of schistosomiasis has been well understood for more than 70 years, it is only recently that it has been possible to analyse the disease at the molecular level and to understand the relationship between the number of parasites in an infected individual and the appearance of overt disease.

SCHISTOSOMIASIS haematobia was discovered in the early 1850s by the great German pathologist Theodore Bilharz when he was performing an autopsy on a young Egyptian male in Cairo. Bilharz papers constitute the classical positions of the most severe changes wrought by this infection upon the urinary tract. "There is catarrhal inflammation of the urinary bladder and ureters. In all cases the inflamed mucous membranes contain an enormous number of ova, while those areas which look normal contain only a few or none. The following were observed as a result of the acute inflammation. (1) Hardening is the most

frequent of the changes. The leather-like changes can be found on every part of the urinary bladder and sometimes cover over half its free surface. They form ring-like deposits in the ureters through which the lumen often is narrowed. In one case there was complete obstruction. The natural consequences of this narrowing is dilatation of that part of the ureter which lies above the narrow area and of the renal pelvis and calices. In the case of complete obstruction the renal parenchyma had vanished completely. (2) Polypoid hypertrophy—the ova of these parasites lie in the parenchyma of the polyps in great numbers, and mainly in a fresh state. (3) Ulcer formation."¹ (Contemporary discussions of these three types of pathology—obstructive uropathy², polyposis³ and ulceration⁴—have been provided by

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Smith *et al.*) "I believe that the immediate cause of the inflammatory processes is the deposition of ova into the capillaries, submucous connective tissue, and the tissue of the mucous membrane in the bladder and of the ureters."¹ Recent studies by Von Lichtenberg *et al.* in Nigeria have described evolution of the pathological processes from early, highly inflammatory lesions known as polypoid patches through the development of lesions with greatly diminished inflammatory infiltrates known as fibrous patches to the final stages made up largely of calcified eggs and some fibrous tissue classically called sandy patches⁵. Quantitative studies by Von Lichtenberg *et al.* and by Cheever *et al.*⁶ in Egypt have revealed relationships between the degree of pathology and the intensity of infection as measured by the numbers of eggs in the tissues, the number of worms in the blood vessels and the number of eggs excreted into the urine.

The classical description of schistosomiasis of the liver was published in 1904 by an English pathologist, William St Clair Symmers, working at the same hospital (Kasr El Ainy) as did Bilharz. "To the naked eye the liver is somewhat enlarged, and the surface shows a peculiar shagreened appearance owing to a pronounced increase of the perivascular connective tissue. On section the liver presents a remarkable appearance due to an enormous increase of fibrous tissue which normally surrounds the portal canals. Microscopically, there is a great increase of the periportal connective tissue, especially of the sublobular and larger portal canals rather than those of interlobular distribution. The fibres are wavy and parallel, for the most part, but in certain places they are ranged concentrically. A small blood vessel may often be seen running up to such a mass of concentrically arranged young fibrous tissue, and lying in the centre is an ovum, as if it had become impacted in the vessel and had by its presence produced the proliferation of tissue which resulted in cirrhosis. This cirrhosis is, I believe, due to the presence of the *Bilharzia ovum* . . ."⁷ At about that period another major form of this disease of the liver with similar pathology was discovered in the Far East and called schistosomiasis japonica. Sometime later pathologists in Egypt⁸ and Brazil⁹ pointed out that the liver disease of schistosomiasis was not a form of cirrhosis but was essentially limited to periportal fibrosis, the patients having relatively little involvement of the liver parenchymal cell. More recent advances include the demonstration of a presinusoidal block to liver blood flow in association with normal total liver blood flow due to pronounced arterialisations of the liver¹⁰. In addition, quantitative relationships have been observed between the degree of pathology, the numbers of eggs in the tissues and worms in the blood vessels and the excretion of eggs in the faeces^{6,11}. Thus, it can be seen that the great investigators of the past provided a true picture of the gross and microscopic pathology of the end stages of schistosomiasis as seen in patients who died in hospitals. While this can be little improved upon today it is only a very small part of the pathobiology of schistosomiasis.

Biology

Billions of people, animals, birds and molluscs suffer from infection with many different species of schistosomes. These so-called digenetic trematodes are organisms which alternate generations between definitive hosts (mammals and birds) in which sexual reproduction takes place, and intermediate hosts (snails) in which asexual multiplication occurs. The worms are known as blood flukes, because their habitat in the definitive hosts is the blood vessels, usually the portal and mesenteric veins, but also the vesical plexuses of the urinary bladder and even the nasal venules (for example, *Schistosoma nasalis* in cattle in India)¹². Within the blood vessels the thread-like female schistosome lies within a cleft in the body of the male worm remaining in a state of 'copulo' for many years during which it produces between 300 and 3,000 eggs daily. The embryo protected by the egg shell develops into a ciliated organism called a miracidium within 6 days and begins to secrete proteolytic enzymes which are emitted through ultramicroscopic pores

in the eggshell. These enzymes enable the egg to pass out of the venules, through the tissues and into the lumen of the intestines, urinary bladder or nose, and thence into the outside environment. Well over 50% of the eggs, however, remain within the body of the definitive host. If the egg enters fresh water, it hatches and the miracidium emerges, swims about and on encountering a susceptible snail species penetrates the soft tissues developing locally into a mother sporocyst which soon produces multiple daughter sporocysts. These organisms migrate from the area of penetration to the digestive gland (hepatopancreas) of the snail. After several weeks, cercariae begin to form. When the forktailed, motile forms reach maturity they issue from the snails either in small numbers or in thousands daily, depending on the species of schistosome. Immersion of definitive hosts in water enables the cercariae to penetrate the intact skin in a matter of minutes by means of both enzymes and vigorous movement. Immediately on entering the skin these organisms change drastically, anatomically, physiologically and biochemically, becoming schistosomula. These young schistosomes remain in the skin for a few days (even in excellent hosts as many as 40% of them die in this site), and then migrate to the lungs via the blood stream and lymphatics. Almost all species then migrate through the blood stream or tissues to the liver where they mature into adult worms, mate and pass to their particular final habitats in the mesenteric, vesical or nasal venules within a period of 4-13 weeks (depending on the species) from the time of penetration. The adult schistosomes do not replicate within the mammalian host, and their average lifespan seems to be between 5 and 10 years.

Pathobiology

As with most infectious diseases, the ability of the schistosomes to produce disease is related to their reproductive capacity. Even where host reactivity plays a considerable part in the pathogenesis of infectious diseases the replication of the virus, bacterium, fungus or protozoan organism to enormous numbers is usually an essential factor in disease production. This holds true for schistosomiasis in the molluscan intermediate host in which infection by a single miracidium results in the development of thousands of cercariae in the hepatopancreas of the snail, with partial or total destruction of that organ. The consequences to the snail are reduced rates of growth, and egg laying and increased mortality. This, and the relatively short life span of normal snails in nature and their enormous reproductive capacity may explain the low prevalence of infection of snails (usually 0.5% or less) even in areas of high prevalence in man.

In the definitive host, including man, however, the schistosomes themselves do not replicate, although each worm pair produces large numbers of eggs daily over a period of many years. A large proportion of the eggs is retained in the host tissues, and they are the cause of the major disease syndromes of schistosomiasis. The total number of eggs entering the tissues is directly related to the numbers of worm pairs. Thus, 60% retention of *S. mansoni* eggs would mean 180 eggs per day for one worm pair, but 180,000 eggs in individuals harbouring 1,000 worm pairs. It is common to all helminths that the adults do not replicate, but the relationship between worm burden and disease is clearest for hookworms. Assuming that each worm removes 0.15 ml of blood daily, iron loss from 1 or even 10 worms is negligible; the loss from 1,000 worms taking 150 ml daily is another matter entirely and iron deficiency anaemia, which is the main hazard of hookworm infection, often ensues¹³. Furthermore, most infected individuals carry only a small number of parasites¹⁴. In many endemic areas fewer than 10% of the population have heavy infections, and even in places with maximal prevalence only about 30% of the population have heavy infections. Field studies in many parts of the world including Uganda¹⁵, St Lucia¹⁶, Kenya¹⁷ and Brazil¹⁸, have shown a relationship between hepatomegaly and splenomegaly and intensity of infection. In areas where prevalence and intensity of infection is low such as present-day Puerto Rico¹⁹

Table 1 Effect of immunosuppressive measures on granuloma formation around schistosome eggs injected into the microvasculature of the lungs of mice (except as noted)

Cell-mediated hypersensitivity		Greater suppression of:	
Measure	Degree of suppression	Measure	Degree of suppression
Niridazole ³⁰	++++	Antineutrophil serum*	0
Cholera toxin ³¹	++++	Anti mu serum*	0
Antilymphocyte serum ³²	++++	Cobra venom factor*	0
Neonatal thymectomy (mice ³³ and chickens ³⁴)	+++	Chronic X-ray irradiation ³⁵	0
Diabetes ³⁵	++++	Bursectomy (chickens) ³⁴	0
Hodgkin's disease (SJL/J MICE) ³⁶	++++	Friend virus leukaemia ³⁶	0
Nude mice ³⁷	++++		

*Manuscript in preparation.

and in Yemeni immigrants in California²⁰ very little liver disease has been detected.

Pathogenesis

The pathogenesis of many of the relatively minor disease syndromes associated with schistosomiasis has both proven and putative immunological associations. The earliest of these is schistosome dermatitis, or swimmer's itch, which is a transient pruritic, papular rash occurring most frequently in insusceptible definitive hosts after penetration of cercariae and their death in the skin¹². This leads to relatively specific sensitisation, and biopsy evidence in man and studies in experimental animals suggests hypersensitivity reactions of both the immediate and delayed types¹². In a small proportion of infected individuals a syndrome known as acute schistosomiasis can be seen; it consists of fever, eosinophilia, splenomegaly, lymphadenopathy and urticaria and is usually found in primary infections shortly after the onset of egg laying by the worms. This reaction has been postulated to be a form of serum sickness due to immune complexes¹². Another form of immune complex disease, glomerulonephritis has been reported to occur in patients with schistosomiasis mansoni, and IgG and IgM complexes have been observed in kidney biopsies¹². Studies in experimental animals have shown the presence of glomerulopathy and its mechanisms are now being explored²¹. The clinical significance of these findings is not known.

Over the past 15 years, however, it has been clearly shown that the major disease syndromes of schistosomiasis, hepatosplenic and obstructive urinary tract disease, are due to hypersensitivity reactions of the host to the schistosome eggs trapped in the tissues²². Although both Bilharz¹ and Symmers⁷ explicitly stated that the schistosome eggs were the cause of the inflammation and fibrosis of urinary and hepatic schistosomiasis, it was not until animal models of the respective disease syndromes were developed in primates and rodents in the last two decades that the eggs were definitively shown to be the cause of the disease syndromes¹². This was most clearly demonstrated in a mouse model of hepatosplenic disease in which the host-parasite relationship was manipulated to eliminate the theories of toxin secretion by the worms and dead adult worms. Only when egg production was present did hepatomegaly, splenomegaly, portal hypertension and esophageal varices occur in the mouse²².

The essential role of the host granulomatous inflammatory response to the eggs in the production of obstruction to liver portal blood flow was then demonstrated by microcirculation studies in living mice²³. In the early stages of infection eggs impacted in the portal venules often did not completely occlude the circulation, and had no effect on the surrounding blood vessels. When the large avascular granulomas, often 100 times the volume of the eggs alone, formed around the eggs, however, not only was local blood flow completely blocked but a large surrounding area was destroyed. Later, a fibrous scar formed and new blood vessels developed. All of the new blood vessels

were arterial; and thus, obstruction to portal blood flow by the granulomatous inflammation and fibrosis eventually leads to the syndrome of portal hypertension, congestive splenomegaly and portal-systemic collateral circulation. Arterialisation of the liver through neovascular formation in the fibrous tissue maintains total liver blood flow within normal limits leading to adequate perfusion of the liver parenchymal cells and the good liver function associated with the classical hepatosplenic syndrome. Studies both in murine models and man have confirmed this view of the pathophysiology of this disease¹².

Investigations of the immunological aetiology of the schistosome egg granuloma were greatly facilitated by the development of a technique in which eggs isolated from the livers of infected animals were injected intravenously into tail veins of mice, dispersing the eggs throughout the pulmonary microvasculature²⁴. The key experiments revealed that secondary exposure to schistosome eggs (following primary exposure by intraperitoneal injection of eggs) resulted in marked anamnestic reactivity—greatly accelerated and augmented granuloma formation²⁵. This reactivity was shown to be specific (there was no cross-reactivity with *Ascaris* eggs) and transferable with lymph node and spleen cells, but not with serum²⁵. Complete specificity was subsequently demonstrated with *S. japonicum* eggs, but there was partial cross-reactivity with *S. haematobium* eggs²⁶. Studies using the same methodology have demonstrated that the granulomatous inflammation around *S. haematobium* eggs shows similar specificity and is again transferable with cells, but not with serum²⁷. Interestingly enough, the response to *S. japonicum* eggs appears to be different: histologically, *S. japonicum* eggs are found in aggregates in the tissues, large eosinophilic abscesses form in the early lesions, and many plasma cells are seen. Granulomatous activity is exceedingly meagre around the single eggs injected into the pulmonary microvasculature even in naturally infected animals²⁸. Furthermore, in early natural infections and following sensitisation with soluble egg antigens only immediate footpad swelling occurs in *S. japonicum* infected mice²⁹ while only delayed footpad swelling occurs in *S. mansoni* and *S. haematobium* sensitised mice. Thus, the *S. mansoni* and *S. haematobium* egg granulomas seem to be largely immunological reactions of the cell-mediated type, but the aetiology of the *S. japonicum* reaction remains unclear, although there are some indications that antibody-mediated inflammatory responses may be occurring in this lesion^{28, 29}.

Extensive studies of the effects of immunosuppressive measures on the *S. mansoni* granuloma strongly support the belief that this lesion is essentially a cell-mediated immunological reaction^{30–38} (Table 1). Further evidence supporting the cell-mediated aetiology of the *S. mansoni* egg granuloma, and illuminating its mechanism is provided by a series of experiments in which intact granulomas were isolated from the livers of infected animals and maintained *in vitro*. Both in the presence and absence of added soluble egg antigens the granulomas have been shown to emit the lymphokines, macrophage migration inhibitory factor³⁹ and eosinophil stimulation promoter⁴⁰. The

lesions contain lymphocytes at their earliest stages, and it is probable that secretion of the above lymphokines and others attract or maintain *in situ* the principal cells of the lesions—macrophages and eosinophils⁴¹. Recent studies using incorporation of radiolabelled leucine have shown production of proteins by the granulomas *in vitro*, but column purification has revealed that these are not immunoglobulins (R. P. Pelley, D. L. Boros & K.S.W., unpublished).

The above findings reveal, therefore, that the schistosome egg is the parasite factor responsible for schistosomal disease, but that the host granulomatous reaction to the eggs is the principal factor in the pathogenesis of schistosomiasis. As the granulomas have been shown to be immunological reactions the chronic syndromes of schistosomiasis are perforce immunological diseases.

In order to carry our knowledge of these diseases to the molecular level it is necessary to isolate and characterise the antigen or antigens from the eggs which are responsible for the granulomatous hypersensitivity. There is now strong evidence that this has been achieved for the *S. mansoni* egg granuloma. Soluble egg antigens (SEA) produced by homogenisation and ultracentrifugation of the eggs have been shown both to induce (by intraperitoneal injection followed by intravenous injection of eggs)⁴² and to elicit (by binding to bentonite particles and intravenous injection)⁴³ granulomatous hypersensitivity. Using serum from chronically infected animals, SEA has been shown to contain three main serological antigens⁴⁴. These antigens have each been isolated in pure form from SEA by desalting on Sephadex G25, affinity chromatography on concanavalin A-Sepharose and ion-exchange chromatography on DEAE cellulose^{44,45}. Major serological antigen₁ (MSA₁), a glycoprotein of molecular weight 50,000 (ref. 46) which is the most abundant of the three antigens, seems to be the principal antigen involved in granuloma formation on the basis of the following evidence. (1) Radioimmunoassay using ¹²⁵I labelled purified antigens and inhibition with crude antigen have shown that MSA₁ has the same highly stage and species specific characteristics as the *S. mansoni* eggs in the induction of granulomatous hypersensitivity⁴⁷; (2) intraperitoneal injection of antigen-antibody complexes prepared from chronic infection serum and purified MSA₁ have resulted in marked sensitisation to subsequent egg injection⁴⁶. (3) The con A-Sepharose eluate induced by α -methyl mannoside was markedly more sensitising than crude SEA while the column drop-through was nonsensitising (data not shown). We are still working on the elucidation of this relationship.

Conclusions

The basic pathology of chronic schistosomiasis as seen at autopsy of patients in urban university hospitals has been established since the discovery of urinary and hepatic schistosomiasis in 1856 and 1904 respectively. Fibrosis induced by inflammation around schistosome eggs trapped in the presinusoidal venules of the liver (hepatosplenic disease) or the tissues of the urinary bladder and ureters (urinary schistosomiasis) leads to obstruction, respectively, of portal blood flow and urine flow. Modern pathology has illuminated the pathophysiology of the syndromes (particularly the alterations in liver circulation) and placed the diseases within a quantitative context in terms of worm and egg burdens.

The pathobiology of schistosomiasis which infects billions of people, animals, birds and snails in rural areas throughout the world reveals the following: (1) the high morbidity and mortality among snails related to multiplication of the schistosomes in the hepatopancreas; (2) the relatively low morbidity and mortality in humans and animals because the schistosomes do not multiply in these definitive hosts; and (3) the relation of disease to intensity of infection in the context of an over-dispersed distribution of the worms in the definitive host population with a relatively small proportion carrying heavy worm burdens.

Studies of the pathogenesis of chronic schistosomiasis in experimental animals have confirmed the beliefs of Bilharz and Symmers that the parasite factor responsible for disease is the eggs trapped in the host tissues. The granulomatous inflammation around the eggs and subsequent fibrosis have been shown to be essential to the development of the disease syndromes and this host response has been found to be an immunological reaction of the delayed hypersensitivity type, revealing that schistosomiasis is essentially an immunological disease. It seems that the major antigen responsible for the disease has been isolated from the eggs, purified and partially characterised.

Thus, the pathology of the chronic disease syndromes of schistosomiasis as originally described in the autopsy room of a university hospital in Egypt 125 and 75 years ago has been placed in the broad context of the biology of the infection in the rural areas of the world. The pathogenesis of schistosomiasis has recently been elucidated in experimental animals, revealing that it is an immunological disease, and the probable isolation of the antigen responsible for this syndrome has brought our knowledge of this major disease of men and animals to the molecular level.

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1. Bilharz, T. *Wien. Med. Wochenschr.* 6, 49–52, 65–68 (1856).
2. Smith, J. H., Kelada, A. S., Khalil, A. & Torky, A. H. *Am. J. trop. Med. Hyg.* 26, 96–108 (1977).
3. Smith, J. H., Torky, H., Kelada, A. S. & Farid, Z. *Am. J. trop. Med. Hyg.* 26, 85–88 (1977).
4. Smith, J. H., Kelada, A. S. & Khalil, A. *Am. J. trop. Med. Hyg.* 26, 89–95 (1977).
5. Von Lichtenberg, F., Edington, G. M., Nwabuebo, I., Taylor, J. R. & Smith, J. H. *Am. J. trop. Med. Hyg.* 20, 244–254 (1971).
6. Cheever, A. W., Kamel, I. A., Elwi, A. M., Mosimann, J. E. & Donnei, R. *Am. J. trop. Med. Hyg.* 26, 702–716 (1977).
7. Symmers, W. St C. *J. Path. Bact.* 9, 237–239 (1904).
8. Hashem, M. *J. Egypt. Med. Ass.* 30, 48–79 (1947).
9. Bogliolo, L. *Ann. trop. Med. Parasitol.* 51, 1–14 (1957).
10. Andrade, Z. A. & Cheever, A. W. *Am. J. trop. Med. Hyg.* 20, 425–432 (1971).
11. Cheever, A. W. *Am. J. trop. Med. Hyg.* 17, 38–64 (1968).
12. Warren, K. S. *Helm. Abs.* 42, 591–633 (1973).
13. Lozoff, B., Warren, K. S. & Mahmoud, A. A. F. *J. infect. Dis.* 132, 606–610 (1975).
14. Warren, K. S. *J. infect. Dis.* 127, 595–609 (1973).
15. Ongom, V. L. & Bradley, D. J. *Trans. R. Soc. trop. Med. Hyg.* 66, 835–851 (1972).
16. Cook, J. A., Baker, S. T., Warren, K. S. & Jordan, P. *Am. J. trop. Med. Hyg.* 23, 625–633 (1974).
17. Siongok, T. K. A. *et al. Am. J. trop. Med. Hyg.* 25, 273–284 (1976).
18. Lehman, J. S., Jr, Mott, K. E., Morrow, R. H., Jr, Muniz, T. M. & Boyer, M. H. *Am. J. trop. Med. Hyg.* 25, 285–294 (1976).
19. Cline, B. L., Rymzo, W. T., Hiatt, R. A., Knight, W. B. & Berrios-Duran, L. A. *Am. J. trop. Med. Hyg.* 26, 109–117 (1977).
20. Warren, K. S., Mahmoud, A. A. F., Cummings, P., Murphy, D. J. & Houser, H. B. *Am. J. trop. Med. Hyg.* 23, 902–909 (1974).
21. Sadun, E. H. *et al. Am. J. trop. Med. Hyg.* 24, 619–631 (1975).
22. Warren, K. S. *Trans. R. Soc. trop. Med. Hyg.* 66, 417–434 (1972).
23. Bloch, E. H., Abdel Wahab, M. F. & Warren, K. S. *Am. J. trop. Med. Hyg.* 21, 546–557 (1972).
24. Von Lichtenberg, F. *Am. J. Path.* 41, 711–731 (1962).
25. Warren, K. S., Domingo, E. O. & Cowan, R. B. T. *Am. J. Pathol.* 51, 735–756 (1967).
26. Warren, K. S. & Domingo, E. O. *Am. J. trop. Med. Hyg.* 19, 292–304 (1970).
27. Kassir, A. I., Warren, K. S. & Mahmoud, A. A. F. *Cell. Immun.* (in the press).
28. Warren, K. S., Boros, D. L., Hang, L. M. & Mahmoud, A. A. F. *Am. J. Pathol.* 80, 279–294 (1975).
29. Warren, K. S., Grove, D. I. & Pelley, R. P. *Am. J. trop. Med. Hyg.* 27, 271–275 (1978).
30. Mahmoud, A. A. F., Mandel, M. A., Warren, K. S. & Webster, L. T. *Jr J. Immun.* 114, 279–283 (1975).
31. Warren, K. S. *et al. J. Immun.* 112, 996–1007 (1974).
32. Domingo, E. O. & Warren, K. S. *Am. J. Pathol.* 52, 613–631 (1968).
33. Domingo, E. O. & Warren, K. S. *Am. J. Pathol.* 51, 757–767 (1967).
34. Davis, B. H., Mahmoud, A. A. F. & Warren, K. S. *J. Immun.* 113, 1064–1067 (1974).
35. Mahmoud, A. A. F., Rodman, H. M., Mandel, M. A. & Warren, K. S. *J. clin. Invest.* 57, 362–367 (1976).
36. Warren, K. S. *Am. J. Pathol.* 56, 293–303 (1969).
37. Phillips, S. M., DiConza, J. J., Gold, Q. A. & Reid, W. A. *J. Immun.* 118, 594–599 (1977).
38. Perrotto, J. L. & Warren, K. S. *Am. J. Pathol.* 56, 279–291 (1969).
39. Boros, D. L., Warren, K. S. & Pelley, R. P. *Nature* 246, 224–226 (1973).
40. James, S. L. & Colley, D. G. *J. reticuloendothel. Soc.* 18, 283–293 (1975).
41. Moore, D. L., Grove, D. I. & Warren, K. S. *J. Pathol.* 121, 41–50 (1977).
42. Boros, D. L. & Warren, K. S. *J. exp. Med.* 132, 488–507 (1970).
43. Boros, D. L. & Warren, K. S. *Immunology* 24, 511–529 (1973).
44. Pelley, R. P., Pelley, R. J., Hamburger, J., Peters, P. A. & Warren, K. S. *J. Immun.* 117, 1553–1560 (1976).
45. Pelley, R. P. *Am. J. trop. Med. Hyg.* 26, Supplement (6), 104–112 (1977).
46. Pelley, R. P. & Pelley, R. J. in *Biochemistry of Parasites and Host Parasite Relationships* (ed. Van den Bossche, H.) 283–290 (North Holland, Amsterdam, 1976).
47. Hamburger, J., Pelley, R. P. & Warren, K. S. *J. Immun.* 117, 1561–1566 (1976).

Antigenic variation in trypanosomes

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In its mammalian host, Trypanosoma brucei is able to change the antigenic character of its glycoprotein surface coat and so evade the host's immune response. This phenotypic change seems to occur spontaneously in 1 in 10,000 individuals but is not due to genetic mutation: host antibody is not necessary for its induction but plays a selective part in bringing about the gross changes in parasite numbers and antigenic character observed in the bloodstream by destroying the main component of what is actually a heterogeneous population. The infecting trypanosome population injected into the mammalian host by the tsetse fly vector may also be heterogeneous. Such heterogeneity complicates plans to vaccinate cattle and people against the African trypanosomes based on the premise that the metacyclic trypanosomes of a clone bear the same surface antigen.

TRYPANOSOMES are tiny flagellated protozoa (Fig. 1) but those that are pathogenic in man and his domestic animals have a powerful hold on the future of Africa. Their presence in lands south of the Sahara thwarts the utilisation of forest grassland for human food production: native vegetation unsuitable for human consumption could be converted by ruminants to high quality protein but cattle are prevented by trypanosomiasis from thriving in these places. The trypanosomes causing the various forms of this disease—*Trypanosoma brucei*, *T. congolense* and *T. vivax*—are transmitted by tsetse flies (*Glossina* spp.) in whose internal organs they undergo a complicated cycle of development before they reach the infective metacyclic stage in the fly's saliva (Fig. 1) (cyclical transmission). For most of this century the main approach to the control of trypanosomiasis has been destruction of these flies and it is hardly surprising that popular belief equates the tsetse rather than the trypanosome with disease. But outside the tsetse belt of Africa essentially similar trypanosomes may be spread by other biting flies or even vampire bats without a cycle of parasite development in the vector. The principal victims of such mechanical transmission are the draught animals of the unmechanised farming world, horses and camels in particular. Human trypanosomiasis, caused by genetic variants of *T. brucei*, is also an important disease as recognised by its inclusion in the World Health Organisation's Special Programme for Research and Training in Tropical Diseases; insofar as we know, it is invariably transmitted cyclically by tsetse flies.

Antigenic variation frustrates vaccination

Disillusion with insecticide spraying and other drastic measures used to prevent the tsetse from infecting man and animal alike with trypanosomes has been but one inducement to look for a suitable way of preventing the mammalian host, once bitten, from developing clinical disease. Vaccination would be preferable to drug prophylaxis (available drugs raise problems of resistance, toxicity and production costs) but such vaccination is at present an unattainable ideal. Attempts to achieve it are frustrated by a remarkable property of the trypanosome—antigenic variation. In chronic infections, the trypanosome population keeps changing its surface antigens; fluctuations in parasitaemia occur repeatedly as the flagellates express an apparently inexhaustible series of variable antigen types (VATs) against each of which the host mounts a new antibody response. The only protection that has been achieved so far is VAT-specific. Understanding the basis of

antigenic change is not only pertinent to the problem of vaccination against important diseases, it also provides a taxing problem to the biologist interested in mechanisms of biological variation.

Fortunately, the African trypanosomes can be adapted to laboratory animals for experimental work on the nature of antigenic variation. Most of this work has been conducted with *T. brucei*, including the sleeping sickness parasites *T. b. rhodesiense* and *T. b. gambiense*. The cattle trypanosomes *T. congolense* and *T. vivax* though economically more important than *T. brucei* are less suitable for basic studies for a variety of reasons. *T. equiperdum*, closely related to *T. brucei* but venereally transmitted between horses in nature, is on the verge of extinction, but transferred by syringe between mice it provides a convenient laboratory model. The development of techniques for cryopreservation of trypanosome populations as defined antigenic material¹ has greatly helped the study of antigenic variation.

One important question is whether antigenic variation is accompanied by a genotypic change in the trypanosome population. Variation occurs in clone infections (see ref. 2 for review), moreover the rate at which new variants appear in the blood of an infected animal could be accounted for by the selection of genetic mutants³. In recent years, however, evidence has been accumulating that the expression of alternative genes underlies the antigenic changes observed rather than changes in the genes themselves. Supporting this view are the observations that each clone of trypanosomes produces a similar series of VATs in different hosts^{4,5} and seems to have a distinctive repertoire of VATs, some but not all of which may be shared with other clones^{6,7}. The inference is that the repertoire corresponds to a particular trypanosome genotype.

A striking argument against a mutation-only theory of antigenic variation came from work on the processional order of VATs in the blood. In 1965 Gray⁴ reported that in his experiments using different hosts, certain VATs were always expressed early in an infection with *T. brucei* and that antibodies to these 'predominant' antigens appeared in the host's serum according to an almost predictable sequence as shown by agglutination reactions. What is more, each set of VATs seemed to have a 'basic antigen' to which the trypanosomes quickly reverted on being cyclically transmitted to a new host to start the procession again. Such predictable reversion is difficult to explain solely in terms of mutation and selection. Similar work on the human sleeping sickness trypanosome (*T. b. gambiense*)⁸ and on *T. equiperdum*⁹ have broadly supported these find-

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ings. The magnitude of the antigenic variation problem was amply illustrated in the *T. equiperdum* work. Using the agglutination reaction, Capbern and colleagues⁸ identified at least 101 different VATs in a serodeme (clone derived collection of VATs) of this species by mouse isolation from chronic rabbit infections. After syringe-transfer of a particular VAT this type was always the first to be detected in the blood but it was invariably followed by the basic antigen and the predictable predominant antigens in sequence; later in the infection it was difficult to recognise order in the VAT series.

In cyclically transmitted trypanosomes Gray⁴ found that



Fig. 2 Transmission electron micrograph of section through surface of body of *T. brucei*. Overlying the plasma membrane (pm) of body and flagellum is the variable antigen-containing surface coat (sc) in all the above (Fig. 1) stages in the life-cycle; the coat is absent from developmental stages in the tsetse fly. Microtubules (mt) under the body plasma membrane. Scale bar, 0.1 μ m. (Micrograph: K. Vickerman.)

the basic antigen was the first to induce an antibody response in the mammal host. This raised an important question: is the basic antigen phenotype present on the metacyclic trypanosomes inoculated by the tsetse fly? If it is then vaccination may be easier than we suppose, for it would be an infinitely simpler matter to immunise animals against the basic antigens of local serodemes than to protect stock or people against the complete VAT repertoires of those serodemes. Unfortunately, the difficulties of working with tsetse-transmitted infections of trypanosomes in the laboratory (usually less than 5% of flies infected produce metacyclic trypanosomes), the minute amount of metacyclic material available for testing, and the lack of adequately defined antigens and antisera—to say nothing of the lack of personnel with the appropriate expertise—have combined to make a satisfactory answer to this question slow in coming.

Variable antigen location in the surface coat

Ten years ago I observed that the metacyclic trypanosomes of *T. brucei* resembled the bloodstream stages (Fig. 1) but differed from other tsetse developmental stages in possessing a 12 nm thick surface coat outside the plasma membrane (Fig. 2). The loss of this coat by bloodstream trypanosomes on entering the fly or culture vessel correlated with the loss of variable antigen observed under these circumstances^{9,10}. I proposed that the coat contained the variable antigen and that it adapted the trypanosome to life in the mammal in that it constituted a replaceable surface protecting the trypanosomes from immune assault. This hypothesis of surface coat function in the mammalian stages of the trypanosome life-cycle has been amply confirmed and substantiated by subsequent work¹¹⁻¹⁴. The variable antigens



Fig. 1 Scanning electron micrographs of *Trypanosoma brucei*, showing the flagellum attached to the body and changes in form. Metacyclic trypanosomes (a) from the salivary glands of the tsetse fly are inoculated into the mammalian host when the fly bites. The trypanosome multiplies in the long slender form (b) in the blood and connective tissues of the mammal. Non-multiplicative short stumpy forms (c) arise from slender forms in the blood; although the stumpy forms can infect the vector, they cannot continue the infection in the mammal. Scale bar, 1 μ m. (Micrographs: L. Tetley.)

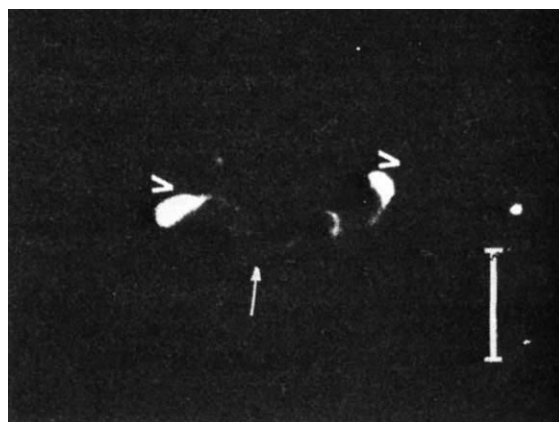


Fig. 3 Mobility of the variable antigen layer on the surface of *Trypanosoma brucei* is shown by 'capping' experiments¹⁴. In the presence of VAT-specific antibody (rat) and fluorescein-conjugated (rabbit) antibody to this immunoglobulin, movement of the variable antigen to the posterior extremity of the trypanosome occurs. The resulting 'cap' is seen here on 2 trypanosomes in contact at their anterior ends (arrow). Scale bar, 10 μ m. (Micrograph: J. D. Barry.)

constituting the coat have been identified and purified^{12,15-17}. Each is a soluble glycoprotein (MW 65,000 as determined by SDS polyacrylamide gel electrophoresis) with oligosaccharide groups which vary in total amount (7-17% w/w) and in their attachment sites along the single polypeptide chain: the proportions of the four sugar components (galactose, glucose, mannose, glucosamine) may also vary¹⁸⁻²⁰. The N-terminal amino acid sequences in the polypeptide chains of different VAT glycoproteins from the same serodeme have no homologous regions²¹—another argument against antigenic variation having a mutational basis. Like the surface glycoproteins of many free-living flagellates those of *T. brucei* seem to be peripheral proteins²² which do not penetrate the lipid bilayer to any significant extent. Lectin-binding and cytochemical studies suggest that much of the carbohydrate is located close to the membrane attachment but whether in all VATs it is unexposed and plays no part in host parasite interactions remains controversial^{18,19,23}. The coat antigen molecules seem to be mobile within the surface membrane as shown by 'capping' experiments (Fig. 3); after antibody induced removal of the antigen the trypanosome can replace its coat from an intracellular store, but as yet ability to replace with a coat of differing VAT has not been demonstrated²⁴.

The purified coat variable antigen glycoprotein is highly immunogenic, 3 µg conferring specific protection on a mouse¹⁹. So, if there is a 'basic' variable antigen and if it could be produced in quantity, protection of larger mammals against cyclically transmitted trypanosomiasis looks feasible. Recently, serial cultivation *in vitro* of mammalian bloodstream *T. brucei* has been achieved for the first time²⁵ so production of variable antigen outside the mammalian host is possible. But we are still left with the questions (i) do the metacyclic trypanosomes of a given serodeme all carry the same antigen in their surface coat, and (ii) is this antigen identical with that believed to be carried by trypanosomes in the first patent parasitaemia in the mammal, that is, the basic antigen? The first attempt at an answer to these questions^{26,27} said "Yes" to the first question and "No" to the second, that is, the trypanosomes revert to a basic antigen at the metacyclic stage but change their VAT shortly after infecting a mammal. Results from the Antwerp Institute of Tropical Medicine and from my own labora-

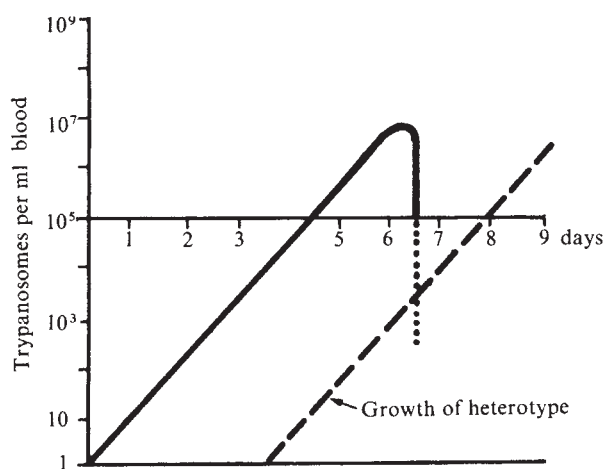


Fig. 4 Theoretical graph to show relationship between antigenic variation and the relapsing parasitaemia in a mouse infected with a single *Trypanosoma brucei* organism. 10^5 represents the level of patency of the parasitaemia; the doubling time of the trypanosome is taken as 6 h. Heterotypes (individuals with a different surface antigen) appear at a ratio of 1 organism to 10^4 homotype organisms²⁸, and the growth of one such heterotype is shown. Remission of the original homotype population on the 7th day is followed by patency of the relapse population arising from this heterotype. (Unpublished graph: W. J. Herbert.)

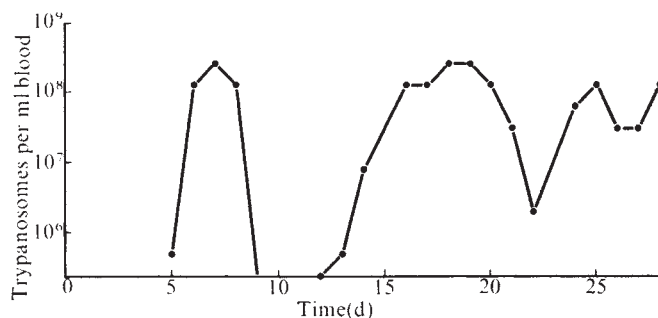


Fig. 5 Actual course of parasitaemia in a mouse infected with a single trypanosome of VAT AnTat 1. The first peak is due to proliferation of AnTat 1 trypanosomes but relapse peaks are less distinct owing to overlapping growth of VATs arising as heterotypes in the manner illustrated in Fig. 3, to possible inhibition of multiplication of some VATs by others, and to release of trypanosomes into the blood from extra-vascular sites. (Unpublished data: J. D. Barry.)

tory cast doubt on the first of these conclusions and prompt us to question the admittedly attractive concept of a basic antigen as a foundation for vaccination plans.

Antigenic variation does not require host antibody

A short while ago²⁸ the emerging picture of antigenic variation was of an orderly programmed sequence of variants with antibody acting as the inducer of antigenic change. This view must now be altered radically as the result of the introduction of techniques which enable us to identify the VAT of individual trypanosomes and so determine precisely the antigenic composition of trypanosome populations. Using VAT-specific immunofluorescence and immune lysis tests, Van Meirvenne and co-workers²⁹ at the Antwerp Institute have demonstrated that an unrelapsed clone population of *T. brucei* is heterogeneous with respect to VAT, minor VATs (heterotypes) being present in addition to the major VAT (homotype) once the parasitaemia has reached patency; any one heterotype may become the homotype of a subsequent population (Figs 4 and 5). Such heterogeneity had previously been postulated by McNeillage, Herbert and Lumsden³⁰ from indirect cloning experiments.

These results suggested that antigenic variation—that is changes in the nature of the glycoprotein surface coat—may be a spontaneous random process capable of producing a large number of VATs in any given population and that host antibody plays a selective and not an inductive part. Some VATs may be produced more readily (or having been produced, be more stable) than others and thus account for the "predominant antigen" effect²⁹. McNeillage and Herbert³¹ had previously produced evidence that some VATs may differ in virulence and that certain VATs may be predominant simply because they overgrow slower-reproducing types. The 'basic antigen' of the first patent parasitaemia could then be the most predominant of the VATs in the serodeme. But small numbers of heterotypes which are undetectable using the agglutination reaction could also be present and should be looked for. It is worth noting that in Gray's experiments⁴ on fly transmission a mixture of VATs was detected in a few initial parasitaemias.

In order to test whether VAT heterogeneity is found in the first parasitaemia of a fly-induced infection or is a feature of syringe-passaged trypanosome lines only, we transmitted Van Meirvenne's syringe-passaged "AnTat" serodeme through the tsetse fly *Glossina morsitans*³² and studied the VAT composition of the ensuing parasitaemia in mice using monospecific antisera. Marked VAT heterogeneity (19 distinct VATs) was found in the first patent



Fig. 6 Diagram to show differentiation of the mammal-infecting metacyclic trypanosomes in the salivary gland of the tsetse fly; flagellates are depicted in sagittal section. The trypanosome multiplies in the epimastigote form (kinetoplast (k) and associated flagellum base in front of the nucleus (n)) while attached by hemidesmosome junctions between its flagellum and the microvillar border of the secretory epithelium which lines the tubular glands. Differentiation of the trypomastigote (kinetoplast behind nucleus) form occurs with detachment of flagellates from their moorings; the single mitochondrion (m) becomes much reduced as its kinetoplast (mitochondrial DNA) shifts to the posterior position and a thick surface coat forms progressively on the plasma membrane. (Based on refs 35 and 36.)

trypanosome population, suggesting that either VAT diversity developed quickly after infection of the mammal or that the metacyclic inoculum from the fly was already heterogeneous. Some of the clones isolated from this early infection proved extremely unstable. For example, with serial 3-day passage through mice (a procedure found satisfactory for conserving the VAT of syringe-passaged clones as 3 days is insufficient time for an effective immune response to develop) one clone was found to consist of 94% heterotypes, 5 passages after typing—further evidence that the immune response plays no necessary part in inducing variation. What, if anything, does control the phenotypic switch remains the biggest mystery of antigenic variation.

So, VAT diversity in *T. brucei* develops quickly after metacyclic infection of the mammal—if it is not already present in the injected metacyclic trypanosomes. And further transmission experiments have shown that this possibility should by no means be discounted.

Diversity of metacyclic antigen types

If all metacyclic trypanosomes of a given serodeme bear the same surface antigen, we would expect them all to react similarly in trypanolysis and immunofluorescence reactions; in our experiments³³ this did not prove to be the case. Thus only a proportion of AnTat serodeme metacyclic trypanosomes from the saliva of *Glossina morsitans* were lysed by rabbit end-infection antiserum (collected 4–6 weeks after

syringe infection with an AnTat clone), by pooled monospecific antisera against 22 different VATs (AnTats 1–22) and by 7-d antisera from animals bitten by AnTat-infected flies. No lysis was seen in controls in which the metacyclics were incubated in fresh guinea pig serum alone or in anti-tsetse saliva antiserum. Absence of lysis in the latter control suggests that the metacyclic surface coat does not incorporate fly salivary components.

Immunofluorescence reactions gave comparable results. That the weakly or non-fluorescing trypanosomes were not metacyclics which had partially acquired (Fig. 6) or partially lost their coat was shown by controls using serum hyper-immune to culture forms of the trypanosome; this serum contained antibodies to the antigens present on the surface of 'uncoated stages' in the tsetse developmental cycle. Reactions have since been obtained between a proportion of metacyclics and monospecific antisera to characterised bloodstream VATs (Fig. 7). These data lead us to conclude that the metacyclics of a *T. brucei* serodeme do not necessarily all carry the same variable antigen on their surface. This finding has important theoretical and practical implications.

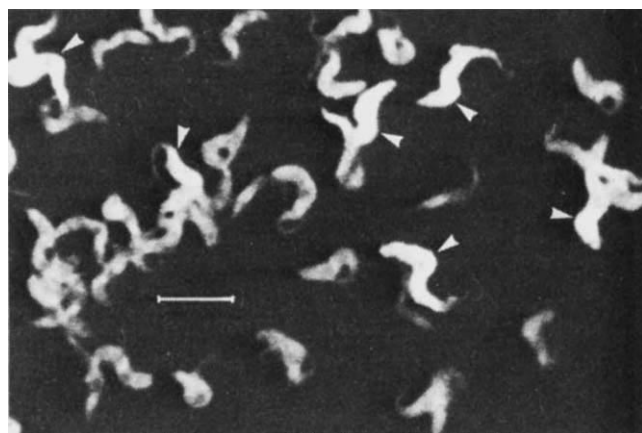


Fig. 7 Heterogeneity of variable antigen type in metacyclic trypanosomes in saliva of *Glossina morsitans*: indirect immunofluorescence reaction conducted on dry saliva using monospecific rabbit antiserum to VAT AnTat 30 and FITC-conjugated goat anti-rabbit Ig³³. Note strongly fluorescing trypanosomes (arrowed) and nonfluorescing individuals which presumably represent other VATs. Scale bar, 10 μ m. (Micrograph: J. D. Barry.)

From the practical viewpoint, metacyclic heterogeneity is consistent with the conspicuous lack of immune protection observed in the field—even in restricted areas where a limited number of basic antigen types might be expected to circulate³⁴. The absence of a single basic antigen per serodeme makes it necessary to revise plans for vaccination dependent on this premise. A careful investigation of the range of metacyclic VATs would be an obvious first step in such a revision.

Confirmation of metacyclic heterogeneity would fully assure us that host antibody is not necessary for the induction of antigenic variation. But if trypanosome antigenic change is purely phenotypic, what could induce VAT heterogeneity? We know that in free-living ciliated protozoa a wide range of environmental changes can trigger expression of a new surface antigen and that the switch over can take a variable number of cell divisions before it is complete: the mammalian host must have an abundance of microenvironments which could induce the expression of different genes in trypanosomes. But in the confines of the

minute salivary glands of *Glossina*, variations in environmental stimuli which could control the nature of the coat acquired (Fig. 6) are not so easy to envisage; amazingly little is known about tsetse physiology in relation to trypanosome development. What may be a novel mechanism of biological variation is begging for further investigation.

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1. Lumsden, W. H. R., Herbert, W. J. & McNeillage, G. J. C. *Techniques with Trypanosomes* (Churchill Livingstone, Edinburgh, 1973).
2. Gray, A. R. & Luckins, A. G. in *Biology of the Kinetoplastida* (eds Lumsden, W. H. R. & Evans, D. A.) 1, 493-512 (Academic, New York, 1976).
3. Seed, J. R. *J. Protozool.* **21**, 639-646 (1974).
4. Gray, A. R. *J. gen. Microbiol.* **41**, 195-214 (1965).
5. Capbern, A., Giroud, C., Baltz, T. & Mattern, P. *Expl Parasit.* **42**, 6-13 (1977).
6. Van Meirvenne, N., Janssens, P. G., Magnus, E., Lumsden, W. H. R. & Herbert, W. J. *Ann. Soc. belge Méd. trop.* **55**, 25-30 (1975).
7. Van Meirvenne, N., Magnus, E. & Vervoort, T. *Ann. Soc. belge Méd. trop.* **57**, 409-423 (1977).
8. Gray, A. R. *Trans. R. Soc. trop. Med. Hyg.* **69**, 131-138 (1975).
9. Seed, J. R. *Parasitology* **54**, 593-596 (1964).
10. Barry, D. J. *Protozool.* **24**, 5A (1977).
11. Vickerman, K. & Luckins, A. G. *Nature* **224**, 1125-1126 (1959).
12. Cross, G. A. M. *Parasitology* **71**, 398-417 (1975).

13. Taylor, D. W. & Cross, G. A. M. *Parasitology* **74**, 47-60 (1977).
14. Fruit, J. *et al. Parasitology* **74**, 185-190 (1977).
15. Allsopp, B. A., Njogu, A. R. & Humphreys, K. C. *Expl. Parasitol.* **29**, 271-284 (1971).
16. Baltz, T., Baltz, D. & Pautrizel, R. *Ann. Immun. Inst. Pasteur* **127C**, 761-774 (1976).
17. Rovis, L., Barbet, A. F. & Williams, R. O. *Nature* **271**, 654-656 (1978).
18. Johnson, J. G. & Cross, G. A. M. *J. Protozool.* **24**, 587-591 (1977).
19. Baltz, T., Baltz, D., Pautrizel, R., Richet, C., Lamblin, G. & Degand, P. *FEBS Lett.* **82**, 93-96 (1977).
20. Allsopp, B. A. & Njogu, A. R. *Parasitology* **69**, 271-281 (1974).
21. Bridgen, P. J., Cross, G. A. M. & Bridgen, J. *Nature* **263**, 613-614 (1976).
22. Vickerman, K. & Preston, T. M. in *Biology of the Kinetoplastida* (eds Lumsden, W. H. R. & Evans, D. A.) 1, 35-113 (Academic, New York, 1976).
23. Cross, G. A. M. & Johnson, J. G. in *Biochemistry of Parasites and Host-Parasite Relationships* (ed. Van den Bossche, H.) 413-420 (Elsevier-North Holland, Amsterdam, 1976).
24. Barry, J. D. & Vickerman, K. *Parasitology* **75**, xxx (1977).
25. Hirumi, H., Doyle, J. J. & Hirumi, K. *Science* **196**, 992-994 (1977).
26. Jenni, L. *Acta tropica* **34**, 35-41 (1977).
27. Jenni, L. *Ann. Soc. belge Méd. trop.* **57**, 383-386 (1977).
28. Vickerman, K. in *Parasites in the Immunized Host: Mechanisms of Survival*. Ciba Fdn Symp. new ser. **25**, 53-70 (1974).
29. Van Meirvenne, N., Janssens, P. G. & Magnus, E. *Ann. Soc. belge Méd. trop.* **55**, 1-23 (1975).
30. McNeillage, G. J. C., Herbert, W. J. & Lumsden, W. H. R. *Expl Parasit.* **25**, 1-7 (1969).
31. McNeillage, G. J. C. & Herbert, W. J. *J. comp. Path.* **78**, 345-349 (1968).
32. Le Ray, D., Barry, J. D., Easton, C. & Vickerman, K. *Ann. Soc. belge Méd. trop.* **57**, 369-381 (1977).
33. Le Ray, D., Barry, J. D. & Vickerman, K. *Nature* **273**, 300-302 (1978).
34. Wilson, A. J. *Trop. Anim. Hlth Prod.* **3**, 14-22 (1971).
35. Vickerman, K. in *Ecology and Physiology of Parasites* (ed. Fallis, A. M.) 58-89 (Toronto University Press, 1971).
36. Steiger, R. F. *Acta tropica* **30**, 64-168 (1973).

Immunity to intestinal parasites

D. Wakelin*

Intestinal parasites are common in man and animals and can cause severe disease. Knowledge of immunity to such infections is limited and comes largely from studies using laboratory host-parasite systems. Understanding how immunity can operate, and why it often does not, is not only of intrinsic interest but necessary for the development of immunological methods of control.

INTESTINAL parasites are probably the most numerous and most widespread of all endoparasitic organisms. Those which occur in man, although not included among the parasitic infections recently designated as areas of special emphasis by WHO, are by their very frequency ($>1 \times 10^6$ cases) of major medical importance, yet little is known of their immunology and immunopathology. In veterinary medicine intestinal parasites are responsible for extensive disease and economic loss.

Much of what is known of immunity to intestinal parasites has come from work with experimental host-parasite systems and this article will be primarily concerned with reviewing the progress such work has made in characterising the immune responses elicited by intestinal protozoa and helminths (parasitic worms) and in identifying the means by which such responses are translated into a protective immunity. Parasites evoke a variety of immune responses, but many of these confer no protection on the host, that is, they exert no deleterious effects on the development or survival of the infection. Protective immunity may be mediated directly by the immune responses themselves, as in the case of intracellular protozoa, or may

additionally require the activity of non-immunological components, as in the helminths (and perhaps the extracellular protozoa). For this reason each category is treated separately, with emphasis on the latter.

Protective immunity against intestinal helminths

Protective responses affect helminths, and thus reduce the pathogenic effects of parasitism, in a variety of ways, but the most dramatic form of immunity is seen when the host expels the worm population from its intestine. This may occur when a larval population is superimposed on an existing adult population, the classical 'self cure' seen in sheep infected with *Haemonchus contortus*, or it may occur during the course of a single primary infection, the 'spontaneous cure' typical of several experimental model systems (Table 1). After self-cure, immunity to reinfection is weak, but after spontaneous cure reinfection induces a greatly accelerated worm loss, sometimes rapid enough to prevent any significant establishment. There is firm evidence that spontaneous cure is induced by thymus-dependent immune responses, being absent or greatly reduced in thymus-deprived or athymic hosts; and in many systems the transfer of immune serum or immune lymphocytes accelerates worm expulsion in recipient animals. Such transfers have formed the basis of much of the experimental analysis of protective immunity.

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Table 1 Spontaneous cure in laboratory infections with helminth parasites

Parasite	Host	Thymus dependency	Transfer of response Serum	Cells	Associated with	
					Reaginic antibody	Inflammation
<i>Nippostrongylus brasiliensis</i>	Rat/mouse	+	+	+	+	+
<i>Trichinella spiralis</i>	Rat/mouse	+	+	+	+	+
<i>Trichuris muris</i>	Mouse	+	+	+	—	—
<i>Trichostrongylus colubriformis</i>	Guinea pig	+	+	+	+	+
<i>Aspiculuris tetraptera</i>	Mouse	+	?	?	—	—
<i>Strongyloides ratti</i>	Rat	+	+	+	?	+
<i>Hymenolepis diminuta</i>	Mouse	+	—	—	—	—

Antibodies

Although immunity can be transferred with immune serum it has proved difficult to correlate protection with circulating antibody levels and attention has been focused on antibodies present at the mucosal surface. Despite the fact that IgA is the dominant class of immunoglobulin in intestinal secretions, specific antiworm IgA has been identified only rarely¹⁻³ and, with one exception, it has not been shown that this immunoglobulin plays a significant part in protective immunity. IgA is known to be effective in the passive transfer of immunity against *Taenia taeniaeformis*, a tapeworm whose larvae enter and penetrate the intestine of the intermediate host, but even here other immunoglobulin classes are also involved^{4,5}. IgA may contribute to protective immunity against *Nippostrongylus brasiliensis*^{3,6} and raised levels of IgA are associated with immunity to *H. contortus*⁷. It has also been suggested that IgA is involved in the expulsion of *Trichinella spiralis* from the rat⁸ as lymphocytes with the characteristics of B blasts transfer immunity somewhat more efficiently than do T cells.

In several helminths, including *N. brasiliensis*⁶ and *Hymenolepis nana*⁹, protective activity has been identified with classes of IgG. Activity of this immunoglobulin against intestinal species implies that some form of pathotopic potentiation (see below) is necessary to allow adequate amounts of antibody to cross the mucosa.

Antiworm antibodies apparently do not cause worm expulsion directly, but may, perhaps by metabolic interference, render worms susceptible to subsequent components of the protective response. Immune suppression of serum recipients prevents worm expulsion^{10,11}, even though the worms still show reduced growth and decreased reproductive potential, and in two experimental systems, *N. brasiliensis* in the rat and *Trichuris muris* in the mouse, it has been shown that such worms can be expelled only if the irradiated host is reconstituted with the appropriate cell population, that is expulsion involves at least two steps^{11,12}. It has been assumed that antibody activity is the direct cause of the cytopathological damage seen in *N. brasiliensis*^{13,14} and *T. spiralis*¹⁵ before spontaneous-cure, but there is no evidence that this is so, indeed with the former, similar damage can be induced by maintenance *in vitro*¹⁶. Recent work with *N. brasiliensis*, which has shown a normal pattern of worm expulsion in mice incapable of significant antibody production¹⁷, has raised the possibility that the first step in worm expulsion may involve humoral factors other than antibody, but the question of whether different host species effect worm expulsion by different mechanisms remains unanswered. It was demonstrated some time ago, for example, that antisera raised in rats infected with *N. brasiliensis* did not transfer immunity against this worm to mice¹⁸.

The most striking antibody response to intestinal nematodes is the production of IgE¹⁹ and it is well known in the case of *N. brasiliensis* that the response involves both the production of specific antiworm IgE and the potentiation of IgE with other specificities^{20,21}. Whether the IgE contributes to worm expulsion is a matter of dispute^{12,22} but it cannot be obligatory as spontaneous cure can occur in its absence^{6,23,24}, and its presence does not guarantee expulsion²⁵.

Lymphocytes

One of the main pathways of lymphocyte traffic is from the gut-associated lymphoid tissues, through the thoracic duct lymph, to the intestinal mucosa. This homing is characteristic of B blasts, destined to become IgA plasma cells²⁶, but can occur also with T blasts²⁷. Homing is known to be enhanced during infection with intestinal nematodes^{8,27,28} and it is therefore not surprising that, in several experimental models, immunity has been successfully transferred using mesenteric lymph node (MLN) cells or thoracic duct lymph (TDL) cells. The populations involved have been identified in certain cases but their functions remain unclear. In *T. spiralis* the most successful transfers in the mouse have been achieved by taking MLN cells during a primary infection, at times when large numbers of T and B blasts are present²⁹, and there is some evidence (D. W., unpublished) that immunity is transferred most effectively by the T cell population, perhaps suggesting a role in activation of accessory cell populations. By contrast, in the rat, using TDL cells from repeatedly immunised donors, it has been shown that, while both T and B populations transfer immunity, the latter bring about expulsion rather more rapidly, suggesting that IgA may play an important part⁸. It is not yet possible to say whether these results reflect a fundamental difference between the two host species in the expulsion of *T. spiralis*, reflect different experimental designs or are simply the experimental isolation of complementary components of a complex response. Certainly it has been suggested that expulsion of *T. spiralis* may involve sequential activity of both antibody and lymphocytes¹⁵.

T cells (Ig⁻ cells) have been implicated as the cells responsible for the expulsion of *N. brasiliensis*, once the worms have been 'damaged' by exposure to antibody³⁰. In an experimental system in which damaged worms were implanted into the intestines of heavily irradiated recipients, expulsion could be induced within 5 d by the transfer of as few as 5×10^7 Ig⁻ TDL cells from immune donors. How these cells mediate expulsion is unknown, but the speed of expulsion and the absence of any concomitant increase in non-lymphoid effector cells suggest some direct antiworm activity. T cells have also been shown to restore the ability to expel *N. brasiliensis* to athymic mice^{31,32}, but this throws no direct light on the functions of the cells, as both the antibody responses to the parasite and the lymphocyte-mediated expulsive step are T-dependent.

Non-lymphoid effector cells

Helminth infections are often associated with marked intestinal inflammation, reflected both in cellular changes in the mucosa itself³³⁻³⁵ and in altered physicochemical conditions in the intestinal lumen^{36,37}. A persistent idea in helminth immunity has been that the immediate cause of worm expulsion lies more in these inflammatory changes than in the immune responses which precede them³⁸; the fact that worms can be expelled from the gut without being killed or even permanently damaged seems to support this view. Inflammation is generated by a complex interaction between immunologically specific components (antibodies, lymphocytes) and nonimmunological, nonspecific components (for example, complement, myeloid cells and biologically active factors) and although complement has not so far

been implicated in worm expulsion¹⁰, there is evidence for the involvement of the other components. Experiments using irradiation and cell reconstitution have shown that expulsion of *N. brasiliensis* and *T. spiralis* occurred only in animals restored with both immune lymphocytes and bone marrow cells^{39,40} and it has been assumed that the latter restore an essential non-lymphoid cell population. Several such populations appear in the inflamed mucosa, but two in particular, the mast cell-basophil series and eosinophils, have been extensively studied^{34,41-47}. In the presence of reaginic antibody and antigen, mast cells degranulate, releasing vasoactive substances which cause increased mucosal permeability⁴⁸. The significance of these events in worm expulsion has generated considerable controversy, too complex to review here, but in essence three main ideas have been proposed, singly or in combination; (1) biogenic amines affect worms directly¹⁰, (2) permeability changes alter the environment to the detriment of the worms⁴⁹⁻⁵¹, and (3) increased permeability allows specific antiworm antibody to reach the worms (pathotopic potentiation)⁵².

Eosinophilia characterises a number of intestinal infections and *in vitro*, eosinophils readily attach to the surface of intestinal nematodes when specific antiserum is present⁵³. These cells are known to release a variety of factors, such as enzymes (for example peroxidases⁵³ and phospholipases⁵⁴) and prostaglandins⁵⁵, which have been considered as possible effectors of worm expulsion, either directly by causing worm damage, or indirectly by altering the intestinal environment. Levels of the enzymes do increase in intestinal helminth infections^{56,54,56} and peroxidases from lamina propria cells are known to kill *T. spiralis in vitro*⁵⁷ but there remains no direct evidence for eosinophil involvement in expulsion and indeed in one case, *T. spiralis* in mice, expulsion is unaffected by eosinophil depletion⁵⁸.

Evidence for the antiworm effects of biologically active factors has been sought from experiments in which such factors (or precursors) are introduced directly into the intestines of infected animals. Although the validity of this approach can be questioned, it has been shown that amines (histamine and 5-hydroxytryptamine) derived from basophils induce expulsion of *Trichostrongylus colubriformis* from guinea pigs and that prostaglandin E induces expulsion of *N. brasiliensis* from rats. In both cases *in vitro* studies have demonstrated a direct effect upon worm metabolism^{59,60}. Prostaglandins do not affect *T. colubriformis* and there is conflicting evidence over the effects of amines on *N. brasiliensis*^{12,22,61}.

If inflammatory changes do cause worm expulsion in an essentially nonspecific fashion, it follows that the changes induced by immune responses against one species of intestinal parasite should affect other species present concurrently. Several studies have shown this to be the case not only with parasites of similar groups (nematodes-nematodes)^{62,63}, but also with nematodes and cestodes⁶⁴ and nematodes and protozoa⁶⁵.

The combination of immunological and nonimmunological components in immunity against helminths may be representative of a common mechanism of protection against extracellular intestinal pathogens, as a similar combination of specific and nonspecific events occurs during the elimination of certain enteric bacteria⁶⁶.

Stimulation of immunity

Intestinal helminths can be potent stimulators of protective immunity. Infections of as few as 10 worms, and even one worm, may elicit measurable protection; larger infections lead to complete and longlasting resistance to reinfection. The major antigens responsible for such immunity are known to be presented by means of the intestine, even when the cycle of the species involves parenteral stages⁶⁷⁻⁶⁹, but the identity and origin of the antigens remains undetermined in the majority of species. As far as nematodes are concerned, it seems likely that the antigens which evoke protective immunity will all prove to be secretory products (possibly enzymes); certainly such antigens

have been identified in the stichosome cells of *T. spiralis*⁷⁰ and *T. muris*⁷¹ and in the excretory glands of *Oesophagostomum radiatum*⁷². The difficulty of isolating protective antigens has hindered the development of successful vaccines and the only effective vaccines so far devised against intestinal helminths (*H. contortus* and *T. colubriformis* in sheep and *Ancylostoma caninum* in dogs) have relied on the use of attenuated living infections^{73,74}.

Failure of protective immunity

Normal spontaneous cure may fail to take place in young animals and there is evidence from experimental studies with *N. brasiliensis* that this failure involves a deficiency in the lymphocyte-mediated component of expulsion^{12,75}. A similar deficiency has been proposed for the failure of immunity against *N. brasiliensis*⁷⁶ and *T. muris*⁷⁷ in lactating rodents. These laboratory findings corroborate field experience with gastrointestinal nematodes in sheep and cattle⁷⁸ and have important implications in the context of the epidemiology and control of such infections. The depression of immunity during lactation can result in contamination of pasture with infective stages at a time when susceptible young hosts are available. The failure of immunity in young hosts leads to the establishment of heavy infection and may, more significantly, render control by vaccination impracticable. It has, for example, been shown that lambs less than six months old can neither acquire immunity to *H. contortus* naturally, nor be immunised by a vaccine effective in older sheep⁷⁹. Lambs fail to show the rise in IgA antibody that follows vaccination in adult sheep⁷ but the correlation of this rise with protection remains to be clarified.

Laboratory studies have also shown that protective immunity is reduced when the host is malnourished⁸⁰, concurrently infected with protozoan⁸¹⁻⁸³ and helminth⁸⁴⁻⁸⁶ parasites, or exposed to repeated low-level infections¹². All these conditions are likely to occur in areas where intestinal helminthiasis are endemic in man and it is more than probable that they contribute to the prevalence and duration of these infections.

In several host-parasite relationships there seems to be no clear-cut protective immunity. There is circumstantial evidence that this may be true of some intestinal helminths in man and ample experimental evidence that this is so in nematode and cestode infections in laboratory animals. A variety of explanations can be proposed: the parasite may, as a result of evolutionary adaptation, show reduced immunogenicity; it may actively suppress immunity against itself; or the host may be genetically incapable of mounting a complete protective response⁸⁷. Analysis of these categories of failure in protective immunity may ultimately prove to be more rewarding than analysis of successful protection.

Protective immunity against intracellular protozoa

Two genera of intracellular intestinal protozoa are of major importance, *Toxoplasma* and *Eimeria*. Only the latter, however, is pathogenic in the intestine and almost all that is known of protective immunity has come from work with species of this genus. Pathogenic infections (notably in poultry) cause extensive mucosal damage as a result of repeated cycles of cell invasion-parasite proliferation-cell invasion⁸⁸. Immune responses are therefore protective if they reduce or prevent proliferation and thus limit the resultant damage. Unlike helminth infections, protective immunity is manifested primarily against challenge infections. Initial infections, as they are essentially self-limiting, with a defined number of asexual cycles followed by a final sexual cycle, are little affected by the immunity they stimulate, although they can be prolonged under immunosuppression⁸⁹.

Immunity against *Eimeria* is characteristically very specific⁹⁰ and there is apparently no nonspecific 'cross-immunity' of the type described above for helminths or of the type described with

other intracellular organisms⁹¹. This, with the intracellular nature of the infection, tends to implicate immune responses as the direct cause of protection, although as yet the precise roles played by components of the immune system are unknown.

In vitro studies have confirmed that specific antibodies exist in the sera of immune animals and show that such antibodies can lyse, agglutinate or reduce the infectivity of the invasive stages (sporozoites, merozoites)⁹⁰. It has proved more difficult to demonstrate that antibodies are involved in immunity *in vivo*, as attempts at passive transfer have often been unsuccessful. A detailed study, using *E. maxima* in chickens, has shown that transfer is possible, but only with sera taken two to three weeks after infection of the donors and given repeatedly during the early stage of infection in the recipients⁹². Sera taken later from donors did not transfer immunity, even though the donors were quite immune, suggesting that serum antibody represents a 'spillover' of locally produced immunoglobulin, perhaps IgA, and is therefore not related to the immune status of the host. An analogue of mammalian IgA does exist in chickens and intestinal extracts containing this immunoglobulin have given protection when tested *in vivo* and against infections established in developing chick embryos^{90,93,94}. Whether antibodies mediate protection *in vivo* by reducing the viability of the invasive stages directly or by perhaps enhancing phagocytosis remains to be determined. It is known that even in immune hosts sporozoites penetrate epithelial cells^{90,95} and with some species, for example *E. maxima*, remain viable for up to 72 h (ref. 95).

Adoptive transfer experiments in chickens have given equivocal results, but the use of infected chick embryos as a test system has shown that lymphocytes from the caecal tonsils of donors immune to *E. tenella* can moderate the course of infection⁹⁶. Recent work with *E. nieschulzi* in rats has shown convincingly that MLN cells and TDL cells will transfer immunity^{97,98} but, in contrast to similar experiments with helminths, large numbers of cells (for example, 6×10^8 TDL cells) are necessary to achieve even 50% protection in recipients. *In vitro*, TDL cells can also cause degenerative changes in sporozoites, but whether this is an antibody-mediated or direct cytotoxicity phenomenon has not been clarified⁹⁷.

Despite the inference that protective immunity is mediated directly by the immune system, there is evidence that infections, both in chickens and rats, stimulate a marked leukocytosis of polymorphonuclear cells, lymphocytes and large mononuclear cells, though not of eosinophils or amine containing cells (ref. 98 and Rose, Hesketh and Ogilvie, personal communication). The rise in cell numbers is antigenically specific and, in chickens, is correlated with cellular infiltration into the lamina propria. The significance of this response is unclear since, although polymorphonuclear cells can phagocytose sporozoites, there is no reduction in the parasite's viability⁹⁹.

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1. Poulain, J., Laffau, G. & Pery, P. *Ann. Immun., Inst. Pasteur* 127C, 215-224 (1976).
2. Cypess, R. H., Ebersole, J. L. & Molinari, J. A. *Int. Archs Allergy appl. Immun.* 55, 496-503 (1977).
3. Sinski, E. & Holmes, P. H. *Expl Parasit.* 43, 382-389 (1977).
4. Musoke, A. J., Williams, J. F., Leid, R. W. & Williams, C. S. F. *Immunology* 29, 845-853 (1975).
5. Lloyd, S. & Soulsby, E. J. L. *Immunology* 34, 939-945 (1978).
6. Jones, V. E., Edwards, A. J. & Ogilvie, B. M. *Immunology* 18, 621-633 (1970).
7. Duncan, J. L., Smith, W. D. & Dargie, J. D. *Vet. Parasit.* 4, 21-27 (1978).
8. Despommier, D. D., McGregor, D. D., Crum, E. D. & Carter, P. B. *Immunology* 33, 797-805 (1977).
9. Di Conza, J. J. *Expl Parasit.* 25, 368-375 (1966).
10. Jones, V. E. & Ogilvie, B. M. *Immunology* 20, 546-561 (1971).
11. Wakelin, D. *Parasitology* 70, 397-405 (1975).
12. Ogilvie, B. M. & Love, R. J. *Transplant Rev.* 19, 147-168 (1974).
13. Ogilvie, B. M. & Hockley, D. J. *Parasit.* 54, 1073-1084 (1968).
14. Lee, D. L. *Parasitology* 59, 29-39 (1969).
15. Love, R. J., Ogilvie, B. M. & McLaren, D. J. *Immunology* 30, 7-15 (1976).
16. Love, R. J., Ogilvie, B. M. & McLaren, D. J. *Parasitology* 71, 275-283 (1975).
17. Jacobson, R. H., Reed, N. D. & Manning, D. D. *Immunology* 32, 867-874 (1977).
18. Ogilvie, B. M. *Int. J. Parasit.* 1, 161-167 (1971).
19. Sadun, E. H. in *Immunity to Animal Parasites* (ed. Soulsby, E. J. L.) 97-129 (Academic, New York, 1972).

20. Orr, T. S. C. & Blair, A. M. J. N. *Life Sci.* 8, 1073-1077 (1969).
21. Jarrett, E. E. & Stewart, D. C. *Immunology* 23, 749-755 (1972).
22. Murray, M. in *Immunity to Animal Parasites* (ed. Soulsby, E. J. L.) 155-190 (Academic, New York, 1972).
23. Wakelin, D. *Parasitology* 57, 515-524 (1967).
24. Behnke, J. M. *Int. J. Parasit.* 5, 511-515 (1975).
25. Jarrett, E. E., Urquhart, G. M. & Douthwaite, R. M. *Expl Parasit.* 24, 270-278 (1969).
26. Guy-Grand, D., Griselli, C. & Vassalli, P. *Eur. J. Immun.* 4, 435-443 (1974).
27. Rose, M. E., Parrott, D. M. V. & Bruce, R. G. *Immunology* 31, 723-730 (1976).
28. Love, R. J. & Ogilvie, B. M. *Expl Parasit.* 41, 124-132 (1977).
29. Wakelin, D. & Wilson, M. M. *Parasitology* 74, 215-224 (1977).
30. Ogilvie, B. M., Love, R. J., Jarra, W. & Brown, K. N. *Immunology* 32, 521-528 (1977).
31. Mitchell, G. F., Hogarth-Scott, R. S., Edwards, R. D. & Moore, T. *Int. Archs Allergy appl. Immun.* 52, 95-104 (1976).
32. Jacobson, R. H. & Reed, N. D. *Int. Archs Allergy appl. Immun.* 52, 160-168 (1976).
33. Larsh, J. E. *Adv. Parasit.* 1, 213-286 (1963).
34. Rothwell, T. L. W. & Dineen, J. K. *Immunology* 22, 733-745 (1972).
35. Taliaferro, W. H. & Sarles, M. P. *J. infect. Dis.* 71, 69-82 (1939).
36. Castro, G. A., Roy, S. A. & Stockstill, R. D. *Expl Parasit.* 36, 307-315 (1974).
37. Castro, G. A., Badial-Aceves, F., Smith, J. W., Dudrick, S. J. & Weisbrodt, N. W. *Gastroenterology* 71, 620-625 (1976).
38. Larsh, J. E. & Race, G. J. *Expl Parasit.* 37, 251-266 (1975).
39. Wakelin, D. & Wilson, M. M. *Parasitology* 74, 225-234 (1977).
40. Dineen, J. K. & Kelly, J. D. *Int. Archs Allergy appl. Immunol.* 45, 759-766 (1973).
41. Ruitenber, E. J., Elgersma, A., Kruizinga, W. & Leenstra, F. *Immunology* 33, 581-587 (1977).
42. Andreassen, J., Hindso, O. & Ruitenber, E. J. *Immunology* 34, 105-113 (1978).
43. Ruitenber, E. J., Leenstra, F. & Elgersma, A. *Br. J. exp. Path.* 58, 311-314 (1977).
44. Jarrett, W. F. H., Jarrett, E. E., Miller, H. R. P. & Urquhart, G. M. in *The Reaction of the Host to Parasitism* (ed. Soulsby, E. J. L.) 191-198 (N. G. Elwert, Marburg, 1968).
45. Wells, P. D. *Expl Parasit.* 12, 82-101 (1962).
46. Kelly, J. D. & Ogilvie, B. M. *Int. Archs Allergy appl. Immun.* 43, 497-509 (1972).
47. Ogilvie, B. M., Mackenzie, C. D. & Love, R. J. *Am. J. trop. Med. Hyg.* 26, 61-67 (1977).
48. Murray, M., Jarrett, W. F. H. & Jennings, F. W. *Immunology* 21, 17-29 (1971).
49. Stewart, A. F. *Aust. J. agric. Res.* 4, 100-117 (1953).
50. Urquhart, G. M., Mulligan, W., Eadie, R. M. & Jennings, F. W. *Expl Parasit.* 17, 210-217 (1965).
51. Mulligan, W., Urquhart, G. M., Jennings, F. W. & Neilson, J. T. M. *Expl Parasit.* 16, 341-347 (1965).
52. Barth, E. E. E., Jarrett, W. F. H. & Urquhart, G. M. *Immunology* 10, 459-464 (1966).
53. McLaren, D. J., Mackenzie, C. D. & Ramalho-Pinto, F. J. *Clin. exp. Immun.* 30, 105-118 (1977).
54. Larsh, J. E., Ottolenghi, A. & Weatherly, N. F. *Expl Parasit.* 36, 299-306 (1974).
55. Hubscher, T. J. *Immun.* 114, 1389-1393 (1975).
56. Ottolenghi, A., Kocan, A. A. & Weatherly, N. F. & Larsh, J. E. *Expl Parasit.* 38, 96-104 (1975).
57. Castro, G. A., Roy, S. A. & Schanbacher, L. M. *J. Parasit.* 61, 1053-1060 (1975).
58. Grove, D. I., Mahmoud, A. A. F. & Warren, K. S. *J. exp. Med.* 145, 755-759 (1977).
59. Rothwell, T. L. W., Prichard, R. K. & Love, R. J. *Int. Archs Allergy appl. Immun.* 46, 1-13 (1974).
60. Richards, A. J., Bryant, C., Kelly, J. D., Windon, R. G. & Dineen, J. K. *Int. J. Parasit.* 7, 153-158 (1977).
61. Rothwell, T. L. W., Love, R. J. & Goodrich, B. S. *Int. Archs Allergy appl. Immun.* 53, 93-95 (1977).
62. Dineen, J. K., Gregg, P., Windon, R. G., Donald, A. D. & Kelly, J. D. *Int. J. Parasit.* 7, 211-215 (1977).
63. Bruce, R. G. & Wakelin, D. *Parasitology* 74, 163-173 (1977).
64. Behnke, J. M., Bland, P. W. & Wakelin, D. *Parasitology* 75, 79-88 (1977).
65. Roberts-Thomson, I. C., Grove, D. I., Stevens, D. P. & Warren, K. S. *Gut* 17, 953-958 (1976).
66. Rowley, D. *Aust. J. exp. Biol. med. Sci.* 55, 1-18 (1977).
67. Ogilvie, B. M. *Parasitology* 55, 325-335 (1965).
68. Vernet, A. in *Biochemistry of Parasites and Host-Parasite Relationships* (ed. Van den Bossche, H.) 319-324 (North-Holland, Amsterdam, 1976).
69. Poulain, J., Pery, P. & Luffau, G. *Ann. Immun., Inst. Pasteur* 127C, 209-13 (1976).
70. Despommier, D. D., Campbell, W. C. & Blair, L. S. *Parasitology* 74, 109-119 (1977).
71. Jenkins, S. N. & Wakelin, D. *Parasitology* 74, 153-161 (1977).
72. Keith, R. K. & Bremner, C. C. *Res. vet. Sci.* 15, 123-124 (1973).
73. Mulligan, W., Gordon, H. M., Stewart, D. F. & Wagland, B. M. *Aust. J. agric. Res.* 12, 1175-1187 (1961).
74. Miller, T. A. *Adv. Parasit.* 9, 153-183 (1971).
75. Love, R. J. & Ogilvie, B. M. *Clin. exp. Immun.* 21, 155-162 (1975).
76. Dineen, J. K. & Kelly, J. D. *Immunology* 22, 1-12 (1972).
77. Selby, G. R. & Wakelin, D. *Parasitology* 71, 77-85 (1975).
78. Ogilvie, B. M. & Jones, V. E. *Prog. Allergy* 17, 93-144 (1973).
79. Urquhart, G. M. *et al. Amer. J. vet. Res.* 27, 1641-1643 (1966).
80. Bolin, T. D., Davis, A. E., Cummins, A. G., Duncombe, V. M. & Kelly, J. D. *Gut* 18, 182-186 (1977).
81. Urquhart, G. M., Murray, M., Murray, P. K., Jennings, F. W. & Bate, E. *Trans. R. Soc. trop. Med. Hyg.* 67, 528-535 (1973).
82. Phillips, R. S., Selby, G. R. & Wakelin, D. *Int. J. Parasit.* 4, 409-415 (1974).
83. Phillips, R. S. & Wakelin, D. *Expl Parasit.* 39, 95-100 (1976).
84. Jenkins, D. C. *Parasitology* 71, 349-355 (1975).
85. Jenkins, S. N. & Behnke, J. M. *Parasitology* 75, 71-78 (1977).
86. Behnke, J. M., Wakelin, D. & Wilson, M. M. *Expl Parasit.* (in the press).
87. Wakelin, D. *Adv. Parasit.* 16 (in the press).
88. Long, P. L. in *The Coccidia: Eimeria, Isospora, Toxoplasma and Related Genera* (eds Hammond, D. M. & Long, P. L.) 254-294 (University Park Press, Baltimore, and Butterworth, London, 1973).
89. Long, P. L. & Rose, M. E. *Parasitology* 60, 147-155 (1970).
90. Rose, M. E. in *The Coccidia: Eimeria, Isospora, Toxoplasma and Related Genera* (eds Hammond, D. M. & Long, P. L.) 295-341 (University Park Press, Baltimore, and Butterworth, London, 1973).
91. Mackaness, G. B. in *Mononuclear Phagocytes* (ed. van Furth, R.) 461-475 (Blackwell, Oxford, 1970).
92. Rose, M. E. *Parasitology* 62, 11-15 (1971).
93. Orland, E. & Rose, M. E. *Immunochimistry* 9, 833-838 (1972).
94. Davis, P. J., Parry, S. H. & Porter, P. *Immunology* 34, 879-888 (1978).
95. Rose, M. E. & Hesketh, P. *Parasitology* 73, 25-37 (1976).
96. Rose, M. E. & Long, P. L. *Parasitology* 63, 299-313 (1971).
97. Liburd, E. M., Armstrong, W. D. & Mahrt, J. L. *Cell. Immun.* 7, 444-452 (1973).
98. Ogilvie, B. M. & Rose, M. E. *INSERM, Paris* 72, 237-248 (1977).
99. Rose, M. E. & Long, P. L. in *Biochemistry of Parasites and Host-Parasite Relationships* (ed. Van den Bossche, H.) 449-455 (North Holland, Amsterdam, 1976).

Cultivation of malarial parasites

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The method for continuous cultivation of Plasmodium falciparum has now been successfully applied to several strains from different geographical areas. It has been used for tests of antimalarial drugs, for studies of parasite-host cell interactions with special reference to sickle haemoglobin, and for the production of amounts of parasite material sufficient for experimental immunisation of Aotus trivirgatus monkeys.

In nature malarial parasites have a complex life cycle consisting of three distinct cycles of reproduction and differentiation. In the mosquito a sexual cycle ends with the formation of infective sporozoites. When these are injected with the bite of the mosquito into a suitable host they initiate a pre-erythrocytic or exo-erythrocytic cycle. In malarial parasites of birds this occurs in reticuloendothelial cells, in those of mammals in hepatic cells. In either case it results in the production of invasive merozoites that begin the erythrocytic cycle, the cycle that produces disease in man. A very small proportion of erythrocytic parasites then develop into gametocytes, forms which produce gametes and initiate the cycle of development in the mosquito.

Studies on the successful cultivation in tissue culture of the exo-erythrocytic forms of bird malaria, on the unsuccessful attempts at propagation *in vitro* of the pre-erythrocytic forms of mammalian malaria, and on the partially successful cultivation of the sporogonic phase with insect tissue cultures, as well as earlier work on short-term maintenance *in vitro* of erythrocytic malaria parasites, have all recently been discussed¹⁻³. Here we shall be concerned only with the continuous cultivation *in vitro* of the erythrocytic stages of *Plasmodium falciparum*, probably the most important of the human malarial parasites⁴.

For the intra-erythrocytic propagation of this organism *in vitro* the following conditions are required—a thin settled layer of human red cells (as obtained, for example, with 1 ml of 10% red cell suspension over an area of 3.3 cm²), a shallow (3-mm) covering layer of culture medium RPMI 1640 containing 25 mM HEPES and 10% human serum of type compatible with the red cells, a gas phase with elevated CO₂ (2–5%) and reduced O₂ (5–18%), either intermittent or slow continuous change of medium, and provision of fresh erythrocytes as the parasite number increases. These conditions can be fulfilled in any of several ways. Probably the simplest is the use of Petri dishes in a candle jar⁵, but this requires manual change of medium at least once a day and more often if one wants a parasitaemia much over 5%. The flow-vial method originally used¹ has been modified to permit continuous maintenance of parasitaemias fluctuating at every cycle (three times per week) from ~1% to ~10%, the reservoir flask of medium being changed once weekly⁶. Other workers have now reported other modifications, but using the same medium and providing the same basic conditions (W. Siddiqui and J. D. Haynes, personal communications).

The culture methods are clearly applicable to most strains of *P. falciparum*—in addition to the Southeast Asian strain (FCR-1) first grown in 1976 we have cultured a South American strain (FCR-2) and two West African strains⁷ (FCR-3, FCR-4). FCR-1 and 2 were started with infected blood from *Aotus* monkeys, whereas FCR-3 and 4 were started directly from infected human blood from patients. Other lines have been started in culture in several other laboratories according to personal communications and reports in abstract form.

The successful maintenance in continuous culture of several different lines of *P. falciparum* immediately raised the following important questions. (1) Could erythrocytes from blood outdated for transfusion purposes be used? (2) Could the human serum be replaced or at least reduced in amount? (3) Would the cultures produce gametocytes? (4) Could the cultures be used for screening *in vitro* of antimalarials? (5) How would the parasites behave in erythrocytes with abnormal haemoglobins or enzyme deficiencies, conditions correlated geographically with hyperendemic malaria⁸? (6) Could high enough parasitaemias be produced *in vitro* to allow collection of adequate numbers of merozoites for research aimed at developing an effective vaccine⁹? We shall now consider briefly what has been learned during the past year and a half with regard to each of these questions.

Erythrocytes and serum

Type A, AB or O erythrocytes have been used successfully after storage in ACD (acid citrate dextrose) or CPD (citrate phosphate dextrose) preservatives at 4 °C for up to 4 weeks, one week past the expiration date for transfusion purposes⁵. A compatible human serum must be provided at 10% (v/v) or 15%. This is obtained from freshly collected blood and must be stored frozen at -20 °C (or lyophilised). Attempts to replace human serum or reduce the concentration to 5% have not so far been successful⁵.

Gametocyte production *in vitro*

As might be expected gametocyte production (Fig. 1a,b) varies with the strain and is best in strains freshly isolated from a patient. The culture conditions we have used are designed to encourage rapid asexual multiplication and are not suitable for detection of gametocytes, which take 10–11 d to mature. Nevertheless immature gametocytes have been detected in two lines even after a year in continuous culture (unpublished observations). That culture conditions could be found that would support formation of mature gametocytes is shown by the recent findings of Carter and Beach¹⁰ who obtained *in vitro* male gametocytes that exflagellated to form male gametes. The exact conditions for bringing this about, however, are not yet known.

Screening antimalarials

The simple Petri dish-candle jar method lends itself readily to work on chemotherapy. We are already using it in studies on chloroquine resistance (P. N. Dinh and W.T., unpublished). Chin has used it to evaluate known antimalarials (reported at 1977 meeting, American Society of Tropical Medicine and Hygiene).

This method permits screening of antimalarials directly against *P. falciparum* in its host cell, the human erythrocyte. In this way we have shown an antimalarial effect of a new type of compound, *S*-isobutyl adenosine, an analogue of adenosyl homocysteine and an inhibitor of methyl transferase¹¹. This method will not eliminate but should greatly reduce the need

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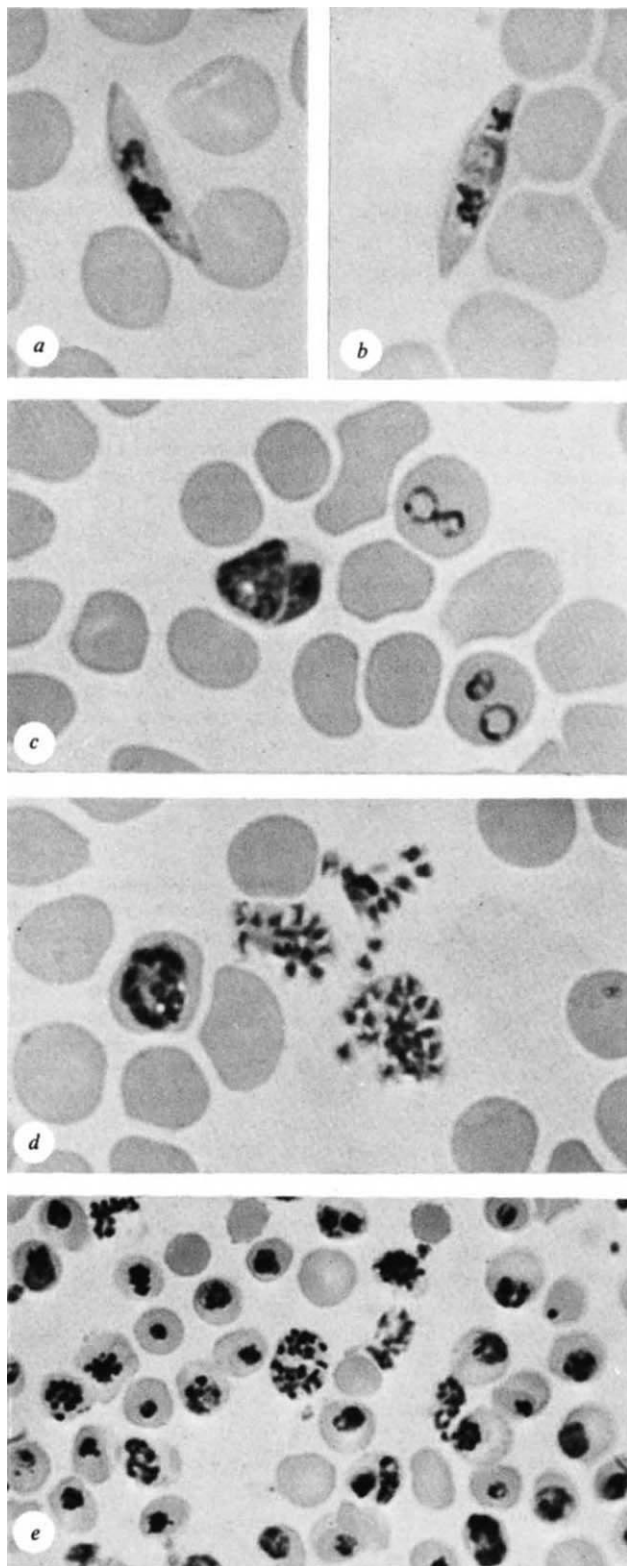


Fig. 1 *Plasmodium falciparum*, stages from cultures *in vitro*. Sexual stages, such as the male (a) ($\times 2,000$) and female (b) ($\times 2,000$) gametocytes appear mature after 8–9 d *in vitro*. They are routinely seen in newly established cultures but their numbers diminish after 4–6 months of continuous culture. Asexual stages such as the rings and trophozoites (c) ($\times 1,600$) and schizonts and merozoites (d) ($\times 1,600$) appear to be normal in every respect even after 18 months of continuous culture. Photomicrographs c and d were taken of cultures of FCR-3/FMG at 20% parasitaemia. This culture was subsequently treated with Physiogel resulting in a concentration of trophozoites, and schizonts to a parasitaemia of 85% (e) ($\times 1,000$). The ring stages and uninfected erythrocytes left over from such a separation procedure can easily be placed back in culture.

for experimental animals, particularly primates, in the development of agents effective against erythrocytic stages of falciparum malaria.

Haemoglobin S

Sickle haemoglobin is the only red cell polymorphism so far sufficiently investigated. Friedman¹² has found that in cultures exposed to 18% O₂ *P. falciparum* develops equally well in cells from homozygous S/S individuals, cells from trait carriers (S/A) and in normal A/A cells. If, however, the O₂ tension is reduced to 5%, a level which occurs physiologically in many tissues of the body, some remarkable differences are seen. Development in A/A cells is just as good as with 18% O₂. In S/S cells sickling occurs and the parasites are quickly destroyed, perhaps in part mechanically by the paracrystalline arrays of sickle haemoglobin. Most interesting, in the heterozygous S/A cells, containing about 40% sickle haemoglobin, the parasites are able to invade and become young rings, but they fail to develop further and die. We have here an explanation at the cellular level for the maintenance, by the selective pressure of malaria, of an otherwise unfavourable gene.

High parasitaemias

The relative parasitaemia (percentage erythrocytes infected) seems to depend partly on the depth of the settled red cell layer, and this in turn depends, for a given surface area, on the packed cell volume (PCV) of the suspension before it has settled. More dilute suspensions in general give a higher relative parasitaemia (Fig. 1c, d) and this is advantageous for some purposes. Absolute numbers of parasites, however, are the same if we have a 10% parasitaemia in a 10% cell suspension or a 20% parasitaemia in a 5% cell suspension. In the continuous flow method we are now using⁶ the parasitaemia quite regularly reaches about 10% three times a week (sometimes 15% or more), starting from about 1% just after addition of fresh red cells. This represents a yield each time of about 10⁹ parasites from a single flow vessel holding originally 12 ml of an 8% red cell suspension. Higher relative parasitaemias (20–30%) in 8% cell suspension have been obtained in large Petri dish cultures with manual change of medium at 8 or 12 h intervals and also in a new semi-automated medium-changing apparatus (J.B.J. and W.T., unpublished). We have used Physiogel (plasma extender) as described by Pasvol *et al.*¹³ or 3% gelatin to concentrate schizonts (R. T. Reese and J.B.J., unpublished). This gives a 60% parasitaemia from an original suspension of only 10% or about 85% from an original level of 25% (Fig. 1e). In this procedure the cells which sediment include cells infected with young rings. This material can be used to start a culture that is highly synchronous for one cycle and yields many merozoites 30 h later. Such preparations rich in merozoites are being used in vaccination experiments with *Aotus trivirgatus* monkeys (R. T. Reese, J.B.J. and W.T., unpublished).

It is clear that the potentialities of the culture method are only beginning to be exploited. Whether it can be applied to *P. vivax* or *P. malariae*, or to the several species of malarial parasites of experimental animals that are of special interest in relation to immunological studies¹ only further work will tell.

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1. *Developments in Malaria Immunology, Tech. Rep. Ser. Wld Hlth Org.* 579 (1975).
2. Beaudoin, R. L. *Bull. Wld Hlth Org.* 55, 373–376 (1977).
3. Vanderberg, J. J., Weiss, M. M. & Mack, S. R. *Bull. Wld Hlth Org.* 55, 377–392 (1977).
4. Trager, W. & Jensen, J. B. *Science* 193, 673–675 (1976).
5. Jensen, J. B. & Trager, W. *J. Parasit.* 63, 883–886 (1977).
6. Trager, W. *Conference on Tropical Medicine, London, 1977* (Royal Society of Medicine, London, in the press).
7. Jensen, J. B. & Trager, W. *Am. J. trop. Med. Hyg.* (in the press).
8. Allison, A. C. *Ann. N.Y. Acad. Sci.* 91, 710–729 (1961).
9. Siddiqui, W. A. *Science* 197, 388–389 (1977).
10. Carter, R. & Beach, R. F. *Nature* 270, 240–241 (1977).
11. Trager, W., Robert-Gero, M. & Lederer, E. *FEBS Lett.* 85, 264–266 (1978).
12. Friedman, M. *Proc. natn. Acad. Sci. U.S.A.* (in the press).
13. Pasvol, G., Weatherall, D. J. & Wilson, R. J. M. *Nature* 270, 171–173 (1977).

Specific and nonspecific immunisation against parasitic infections

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Parasitic diseases present major problems to man and his domesticated animals but do not respond easily to conventional vaccination procedures. Vaccines including attenuated stains, killed parasites, homogenates and soluble extracts all produce varying degrees of protection but controlled infections and immunisation with heterologous species or nonspecific antigens also induce protective immunity.

PARASITIC infections constitute the major health hazard to man in the tropics and sub-tropics, a smaller but real hazard elsewhere and a continual threat to the wellbeing of domesticated animals in all parts of the world. The success of vaccines in the control of many viral and bacterial diseases has not been emulated in the parasitic infections and this is due in part to the fact that most parasites have evolved ways of not only living with the immune response of the host but in many cases circumventing it^{1,2}. Many see the development of a vaccine against any of the major parasitic diseases of man as being one of the greatest challenges in medicine today and the amount of research time devoted to this end is prodigious. This challenge is comparable to the search for vaccines against cancers for in many ways the problems are similar and it may be that some of the solutions will be too.

The subjects of immunity to parasitic infections and immunisation are intertwined for there is a firm belief that no successful vaccine can be developed until a particular immune response is fully understood. Immunity to parasitic infections has, therefore, been the subject of many symposia and books³⁻⁵ and it is my intention in this review simply to summarise the findings of the past 2 years or so with particular reference to those diseases of medical and veterinary importance and their laboratory counterparts.

Acquired immunity to parasites is the rule but it is usually slow to develop and often incomplete. The object of vaccination procedures is not so much to mimic nature but to improve on it because the natural acquired immune response is frequently associated with immunopathological damage and the persistence of parasites in the immune host¹. It is therefore essential to untangle those antigens that are responsible for protection from those that cause immunopathological changes and to eliminate residual parasites that might act as sources for further infections.

The ideal vaccine would be an extract of a parasite consisting of the part that actually stimulates the protective immune response and preferably chemically characterised so that it could be synthesised and used either on its own or in association with a carrier molecule. This ideal has not yet been achieved and even those vaccines that are most successful against viral and bacterial diseases usually consist of inactivated or attenuated whole organisms. Such vaccines have been widely tried in parasitic infections but so intransigent are the problems that many other less orthodox methods of immunisation have also been attempted.

Attenuated parasites

Among the earliest bacterial vaccines were attenuated strains consisting of live organisms that lived long enough to stimulate an immune response but not so long as to cause disease. The complex life cycles of many parasites have led investigators to consider the use of invasive stages

incapacitated so that the transformation into the later more damaging stages cannot occur. This approach has met with considerable success particularly in helminth infections in which the invasive stages have been irradiated. The most striking success has been a vaccine against the cattle lung-worm nematode *Dictyocaulus viviparus*⁶. This commercially available vaccine, consisting of irradiated larvae, elicits a long-lasting immunity against a disease which only 20 years ago killed one-third of infected cattle in Britain. Similarly successful vaccines have been developed for use against *Dictyocaulus filaria* in sheep⁷ and dog hookworm. *Ancylostoma caninum*⁸, but the latter has not been widely used for reasons unrelated to its immunological properties⁹.

Irradiated larvae have also been used experimentally against a number of other nematodes including *Haemonchus contortus* in adult sheep¹⁰ but not lambs¹¹, *Trichostrongylus colubriformis* in sheep and some lambs¹², *Brugia malayi* in monkeys¹³, *Dirofilaria immitis* in dogs¹⁴ and *Litomosoides carinii* in rats¹⁵. Irradiated eggs have also been shown to protect fowl against *Capillaria obsignata*¹⁶.

Considerable success has been achieved in immunising animals against *Schistosoma* spp. using cryopreserved irradiated cercariae or schistosomula larvae^{17,18}. The immunisation of domesticated animals against schistosomiasis is now a real possibility and successful field trials have been carried out with *Schistosoma mattheei* in sheep¹⁹ and *S. bovis* in cattle and sheep²⁰. On the other hand, this technique has met with little success in the immunisation of sheep against *Fasciola hepatica* and only partial success in cattle²¹.

Among the protozoa, irradiated sporozoites have been widely used to induce immunity against a number of malaria parasites²² and in the few trials with humans there have been both limited successes^{23,24} and failures²⁵. Irradiated oocysts of coccidia also confer immunity but this is probably no more than would be expected to follow the ingestion of a small number of untreated oocysts²⁶. Mice can be protected against *Toxoplasma gondii* with irradiated trophozoites²⁷ and partially protected against *Trypanosoma cruzi* with irradiated amastigotes or trypomastigotes²⁸.

Attenuation can also be brought about by maintaining the parasite in culture or in an abnormal host. Avirulent embryoculture attenuated strains of *Elmeria* spp. protect chickens against virulent strains²⁹, cultured *Theileria parva* protects cattle against tick-transmitted infection³⁰ and a hamster-adapted strain of *Schistosoma mattheei* protects sheep against the parent strain³¹.

Killed parasites and homogenates

In general, killed parasites or homogenates are less effective at inducing a protective immune response than living ones and at present such vaccines also suffer from the combined disadvantages of the need for adjuvants and large amounts of parasite antigen, much of which is irrelevant to the protective immune response. There is no doubt that it is possible to induce some degree of protection against most,

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if not all, parasites by the administration of whole organisms or homogenates but this must anticipate the use of smaller amounts of well characterised antigens. Examples of the protection that has been obtained in this way can be selected from the nematodes *Trichostrongylus colubriformis*³² and *Trichuris muris*³³, the trematodes *Schistosoma mansoni*³⁴, although the immunity is not nearly as good as that induced by irradiated cercariae, and *Fasciola hepatica*³⁵ and the cestodes, *Taenia taeniaeformis*³⁶ and *T. saginata*³⁷. In most studies of this kind, the initial success has been followed by attempts to isolate and purify the actual antigens involved and this approach looks as if it could be quite successful although at the present time the protection obtained is often erratic and the results inconclusive.

Whole or homogenates of protozoa also induce protective immunity and a most promising vaccine is one consisting of the merozoites of malaria parasites³⁸. This vaccine is effective against *Plasmodium knowlesi* in monkeys³⁹ and the human parasite *P. falciparum* in owl monkeys^{40,41}, is stable and immunogenic after freezing or freeze drying⁴² but suffers at present from the disadvantage that it requires Freund's complete adjuvant for efficacy.

Vaccines prepared from whole or disrupted cultured parasites are partially protective against *Trypanosoma cruzi* in mice⁴³⁻⁴⁵ but patent or subpatent infections result after challenge. Protection against *T. cruzi* can also be brought about by killed bloodstream trypanosomes with saponin as an adjuvant⁴⁶. Homogenates and fractions of *Trypanosoma brucei* and *T. gambiense* also elicit protective immunity⁴⁷ but equally effective antigens can be extracted from the surface coat⁴⁸ and none of these vaccines overcome the problem of antigenic variation⁴⁹.

Soluble parasite antigens

The use of parasite extracts has many advantages over the use of whole parasites and in several cases such extracts offer real hope of effective and safe vaccines. Highly immunogenic substances, probably enzymes, have been extracted from the anterior glands of several nematodes and in the most extensively studied species, *Trichinella spiralis*, microgram quantities are capable of protecting mice against subsequent challenge⁵⁰. Similar results have been obtained with an extract of the stichosome of *Trichuris muris*³³. Soluble antigens of *Ascaris suum*⁵¹ and *Trichostrongylus colubriformis*⁵² are protective in the guinea pig.

The secretions of larval cestodes also provide a source of protective antigens. Antigens isolated from the culture media of larvae grown *in vitro* protect calves against *Taenia saginata*⁵³ and lambs against *T. ovis*⁵⁴. Secretions that might act as protective antigens have also been identified from *Fasciola hepatica*⁵⁵.

The best studied and characterised protozoal antigens are those of the surface coat of the African trypanosomes particularly *Trypanosoma brucei*^{48,49}. These antigens are extremely immunogenic, their biochemical characteristics are understood⁴⁸ and they could conceivably be artificially synthesised. Unfortunately, the phenomenon of antigenic variation⁴⁹ makes the development of an effective vaccine a near impossibility. The potential of antigens extracted from other protozoa is difficult to assess but soluble antigens found in the serum of monkeys infected with *Plasmodium knowlesi* do confer some immunity⁵⁶.

Controlled infections

The problems associated with developing effective vaccines against parasitic infections, particularly those caused by protozoa, have led many to question the need for conventional vaccines and to contemplate the advantages of letting animals develop a natural immunity after either exposure to a small number of parasites or a drug-controlled infection. Such a deliberate practice is unlikely to gain favour in

human medicine but, in the veterinary field, it has considerable potential particularly for coccidiosis and piroplasmosis. Infections with *Eimeria* spp. are normally self-limiting⁵⁸ and there is abundant evidence that small numbers of oocysts given over a period of time quickly establish a solid immunity^{56,57}. Such controlled infections produce better immunity than infection plus chemoprophylaxis⁵⁸.

The administration of parasitised blood renders cattle resistant to subsequent tick transmitted *Babesia argentina* infections⁵⁹ but this depends on a quirk in the biology of these organisms not yet understood. However, the success of this procedure has led to the use of parasitised blood as a commercial vaccine. Infection with *B. argentina* or *B. bigemina* and concurrent treatment with the drug amido-carb also results in effective protective immunity⁶⁰. Cattle can be immunised against theileriasis, in particular, east coast fever caused by *Theileria parva*, by deliberately infecting them and treating them at the same time with oxytetracycline: field trials suggest that this method is very effective^{61,62}. This procedure is also effective against *T. annulata*⁶³.

Combinations of infection and cure have also been shown to result in the immunisation of rodents against *Trypanosoma brucei* and *T. congolense*⁶⁴ and individual cattle against trypanosomiasis but an even better herd immunity develops if animals are allowed to become infected naturally and only those actually showing symptoms treated with drugs⁶⁵.

Controlled infections also offer some hope of immunisation against leishmaniasis. Immunity to *Leishmania tropica* can be induced in laboratory animals by injecting small numbers of parasites after which a solid immunity develops^{66,67}. Subcutaneous injections of *L. donovani* result in reduced visceral infections after challenge in hamsters⁶⁸. Laboratory experiments with nematodes suggest that controlled infections resulting from a combination of small doses of infective larvae and drug treatment can induce a considerable degree of immunity⁶⁹.

Heterologous protection

Heterologous protection is an extension of the ideas of attenuated and controlled infections and is based on observations that exposure to avirulent parasites may protect animals against challenge with virulent parasites of a different species. Many of the observations on heterologous immunity have been made on mice in which *Plasmodium chabaudi* protects against *P. vinckei*⁷⁰, *Babesia microti* and *Anthemiosoma garnhami* protect against *B. rodhaini* and *P. vinckei*⁷¹ and *Schistosoma bovis* protects against *S. mansoni*⁷². The possibility of using a heterologous parasite to protect against an important disease of domesticated animals emerges from the observation that *Ornithobilharzia turkistanicum* protects cattle against *Schistosoma bovis*⁷³. Some free-living nematodes have been used to protect laboratory animals and cattle against parasitic nematodes; *Rhabditis axei* protects against *Dictyocaulus* spp. in guinea pigs and *Aphelenchus avenae*, *Ditylenchus destructor* and *Neoaplectana glaseri* protect against *Ascaris*⁷⁴ and *R. axei* increases the resistance of lambs to *Dictyocaulus filaria*⁷⁵.

Of possible significance to human medicine is the fact that heterologous immunity exists between species of *Leishmania* and that the relatively avirulent species, *L. guyanensis*, protects against the virulent *L. brasiliensis*⁷⁶.

Nonspecific immunisation

The difficulties inherent in immunising animals and man against parasitic infections coupled with the knowledge that limited immune responses do occur has led, as in tumour immunology, to a search for substances that might stimulate a sluggish or ineffective response. Two separate lines of approach have been adopted; the possibility of using such substances as adjuvants with parasite antigens (immunopotential) and the possibility that they might act non-specifically in the absence of parasite antigens. One of the

most widely used substances that stimulates nonspecific immunity is the *Bacillus Calmette Guérin* vaccine (BCG) which has been extensively used in tumour immunology. The most dramatic effect of BCG is in infections with *Babesia* spp. in rodents against which it completely protects even months after immunisation^{77,78}. BCG also protects, to some extent, against *Plasmodium* spp. in mice⁷⁷. There are differing reports on the efficacy of BCG against *Trypanosoma cruzi*, it being protective in some circumstances⁷⁹ but not in others^{80,81}. BCG protects mice against *Leishmania tropica*⁸² and *L. donovani*⁸³. Among the helminths, BCG has a suppressive effect on the development of *Echinococcus multilocularis* in cotton rats⁸⁴ and *E. granulosus* in jirds⁸⁵ and increases the resistance of mice to *Schistosoma mansoni*⁸⁶. *Corynebacterium parvum* also protects mice against *Babesia* spp. and *Plasmodium* spp.⁸⁷, *Trypanosoma cruzi*^{88,89} and infections with avirulent *Toxoplasma gondii*⁹⁰.

The success of nonspecific immunisation with BCG or *C. parvum* varies according to the nature of the antigen, the way it is administered and the interval between immunisation and challenge. It is also affected by the strain of animal used (unpublished observations) which may be one of the reasons that the immunisation of calves with BCG fails to protect them against *Babesia divergens*⁹¹.

The results obtained using nonspecific immunisation have, so far, been very encouraging. The protection is as good as, or better than, that obtained in conventional ways and the vaccine presents few risks and does not require large amounts of parasite antigen or adjuvants. The possibilities of other nonspecific stimulators of immunity are real and these may include substances better than BCG or *C. parvum*. *Brucella*, *Coxiella*, *Listeria* and *Salmonella* for example, all protect mice against *Babesia* spp. (I. A. Clark, personal communication).

Future prospects

All the accumulated evidence suggests that, difficult as it may be, it should not be impossible to produce a vaccine against any parasitic disease. The important question is whether or not the development of a particular vaccine is likely to be worthwhile even if it can be shown to be safe and effective. There can be no possibility of another success like smallpox vaccine and any anti-parasitic vaccine will be expensive to produce, difficult to administer and unlikely to eradicate any disease.

For diseases in which chemoprophylaxis is possible, there is little immediate need for a vaccine. For coccidiosis in battery chickens, for example, there are cheap and effective drugs that are easy to administer continuously. For cattle trypanosomiasis, the development of a vaccine seems hardly worthwhile in view of the antigenic variation that occurs in species such as *Trypanosoma brucei* and the exposure of cattle to infected tsetse flies and treatment only when they are ill produces strong and effective immunity at minimum expense. The use of drug-controlled infections in theileriasis is another example of an unconventional immunisation procedure against a disease which shows few signs of responding to conventional vaccines. In diseases of veterinary importance, such as these, it may be better to improve these unconventional methods of immunisation than to attempt to develop new ones although the possibilities of drug resistance and toxicity to the consumer will keep alive the eventual need to develop vaccines.

For diseases such as cattle lungworm, effective vaccines are available but it will be important to take stock soon and ask if the parasites that can be protected against in this way are the exception or the rule. If the exception, one might look in vain for a new *Dictyocaulus viviparus* or *Ancylostoma caninum* vaccine and such findings as that vaccines effective against *Haemonchus contortus* in sheep are ineffective in lambs in which they are required should

perhaps direct attention towards alternative control measures.

Nonspecific immunisation against parasitic infections must be regarded as a possible alternative to specific immunisation and there is evidence from laboratory studies that such immunisation procedures may be at least as effective as specific ones. The main advantage of nonspecific immunisation is that the substances that induce the protective response can potentially be manufactured synthetically. As this kind of approach is being developed in the field of tumour immunology, parasitologists may have to be a little patient and follow the lead of tumour immunologists.

There are diseases for which drugs provide little hope and nonspecific immunisation shows few signs of being effective and these are the areas for the development of conventional vaccines. Crude homogenates and extracts are unlikely to be acceptable and the main tasks will be first the isolation of protective antigens and subsequently their artificial synthesis. As the purified substances are unlikely to be highly immunogenic, new and safe adjuvants will have to be developed and, here again, tumour immunology is leading the way.

The future of immunisation against parasitic infections looks bright even if the future for conventional vaccines is less encouraging. In the near future, there will be opportunities for both the rational and the empirical approach but more than anything else there will be the need for wisdom—the wisdom to know when to consider the development of a vaccine and when not to.

1. CIBA Fdn Symp. 25 (1974).
2. Ogilvie, B. M. & Wilson, R. J. M. *Br. med. Bull.* 32, 177–186 (1976).
3. Cohen, S. & Sadun, E. H. (eds) *Immunology of Parasitic Infections* (Blackwell, Oxford, 1976).
4. Miller, L. H., Pino, J. A. & McKelvey, J. J. (eds) *Immunity to Blood Parasites of Animals and Man* (Plenum, New York, 1977).
5. Warren, K. S., David, J., Neva, F. A. & Phifer, K. O. (eds) *Am. J. Trop. Med. Hyg.* 26 Part 2 (Suppl.), 5–249 (1977).
6. Jarrett, W. F. H., Jennings, F. W., McIntyre, W. I. M., Mulligan, W. & Urquhart, G. M. *Immunology* 3, 145–151 (1960).
7. Mulligan, W. in *Nuclear Techniques in Animal Production and Health* 413–419 (I.A.E.A., Vienna, 1976).
8. Miller, T. A. *Adv. Parasit.* 9, 153–183 (1971).
9. Miller, T. A. *Adv. Parasit.* 16, (in the press).
10. Mansfield, M. E., Ozerol, N. H., Courter, M., Green, C. & Levine, N. D. *Am. J. vet. Res.* 35, 1423–1428 (1974).
11. Urquhart, G. M. *et al. Am. J. vet. Res.* 27, 1641–1643 (1966).
12. Dineen, J. K., Gregg, P. & Lascelles, A. K. *Int. J. Parasit.* 8, 59–63 (1978).
13. Wong, M. M., Fredericks, H. J. & Ramachandran, C. P. *Bull. Wild Hlth Org.* 40, 493–501 (1969).
14. Wong, M. M., Guest, M. F. & Lavoipierre, M. J. *Expl Parasit.* 35, 465–474 (1974).
15. Gangadhar-Rao, Y. V. B., Mehta, K. & Subrahmanyam, D. *Expl Parasit.* 43, 39–44 (1977).
16. Ziegler, K. *Acta veterinaria Brno* 44, 115–122 (1975).
17. Taylor, M. G. in *Nuclear Techniques in Helminthology Research* 165–173 (I.A.E.A., Vienna, 1975).
18. James, E. R. & Farrant, J. *Trans. R. Soc. trop. Med. Hyg.* 71, 498–500 (1977).
19. Taylor, M. G. *et al. J. Helminth.* 50, 1–9 (1976).
20. Taylor, M. G. *et al. in Immunology in Parasitic Diseases* (eds Capron, A., Lambert, P.-H., Ogilvie, B. H. & Péry, P.) 291–305 (INSERM, Paris, 1978).
21. Nansen, P. *Res. vet. Sci.* 19, 278–283 (1975).
22. Richards, W. H. G. *Trans. R. Soc. trop. Med. Hyg.* 71, 279–280 (1977).
23. McCarthy, V. C. & Clyde, D. F. *Expl Parasit.* 41, 167–171 (1977).
24. Clyde, D. F., McCarthy, V. C., Miller, R. M. & Woodward, W. E. *Am. J. trop. Med. Hyg.* 24, 397–401 (1975).
25. Bray, R. S. *Trans. R. Soc. trop. Med. Hyg.* 70, 258 (1976).
26. Rose, M. E. *Vet. Rec.* 98, 481–483 (1976).
27. Seah, S. K. K. & Hucal, G. *Can. J. Microbiol.* 21, 1379–1385 (1975).
28. Hanson, W. L., Chapman, W. L. & Waits, V. B. *Int. J. Parasit.* 6, 341–347 (1976).
29. Long, P. L. & Millard, B. J. *Avian Path.* 6, 77–92 (1977).
30. Gill, B. S., Bhattacharyulu, Y., Kaur, D. & Singh, A. *Nature* 264, 355–356 (1976).
31. Dargie, J. D. *et al. J. Helminth.* 51, 347–357 (1977).
32. Rothwell, T. L. W. & Love, R. J. *Int. J. Parasit.* 4, 293–299 (1974).
33. Jennings, S. N. & Wakelin, D. *Parasitology* 74, 153–161 (1977).
34. Murrell, K. D., Dean, D. A. & Stafford, E. E. *Am. J. trop. Med. Hyg.* 24, 955–962 (1975).
35. Lang, B. Z. & Hall, R. F. *J. Parasit.* 63, 1046–1049 (1977).
36. Kwa, B. H. & Liew, F. Y. *J. exp. Med.* 146, 118–131 (1977).
37. Gallie, G. J. & Sewell, M. M. H. *Trop. Anim. Hlth Prod.* 8, 233–242 (1976).
38. Mitchell, G. H. *Trans. R. Soc. trop. Med. Hyg.* 71, 281–282 (1977).
39. Butcher, G. A., Mitchell, G. H. & Cohen, S. *Immunology* 34, 77–86 (1978).
40. Mitchell, G. H., Butcher, G. A., Richards, W. H. G. & Cohen, S. *Lancet* i, 1335–1338 (1977).
41. Siddiqui, W. A. *Bull. Wild Hlth Org.* 55, 403 (1977).
42. Mitchell, G. H., Butcher, G. A., Langhorne, J. & Cohen, S. *Clin. exp. Immun.* 28, 276–279 (1977).
43. Gonzalez Cappa, S. M., Cantarella, A. I., Lajmanovich, S. & Sagura, E. S. *J. Parasit.* 62, 130–131 (1976).
44. Menezes, H. *Tropenmed. Parasit.* 27, 418–421 (1976).
45. McHardy, N. & Elphick, J. P. *Int. J. Parasit.* 8, 25–31 (1978).

46. Neal, R. A. & Johnson, P. *Acta trop.* **34**, 87-96 (1977).
47. Powell, C. N. *Med. J. Zambia* **10**, 27-32 (1976).
48. Cross, G. A. M. *Parasitology* **71**, 393-417 (1975).
49. Vickerman, K. *Nature* **273**, 613-617 (1978).
50. Catty, D. in *Immunology of Parasitic Infections* (eds Cohen, S. & Sadun, E. H.) 359-379 (Blackwell, Oxford, 1976).
51. Stromberg, B. E. & Soulsby, E. J. L. *Int. J. Parasit.* **7**, 287-291 (1977).
52. Rothwell, T. L. W. *Int. J. Parasit.* **8**, 33-37 (1978).
53. Rickard, M. D. & Adolph, A. J. *Vet. Parasit.* **1**, 389-392 (1976).
54. Rickard, M. D. & Adolph, A. J. *Parasitology* **75**, 183-188 (1977).
55. Collins, W. E., Contacos, P. G., Harrison, A. J., Stanfill, P. S. & Skinner, J. C. *Am. J. trop. Med. Hyg.* **26**, 373-376 (1977).
56. Joyner, L. P. & Norton, C. C. *Parasitology* **72**, 115-125 (1976).
57. Hein, H. E. *Expl Parasit.* **40**, 250-260 (1976).
58. Huchermeyer, F. W. J. S. *Af. Vet. Ass.* **47**, 253-254 (1976).
59. Emmerson, F. R., Knott, S. G. & Callow, L. L. *Aust. vet. J.* **52**, 451-454 (1976).
60. Todorovic, R. A. & Tellez, C. H. *Trop. Anim. Hlth Prod.* **7**, 125-131 (1975).
61. Uilenberg, G., Silayo, R. S., Mpangala, C., Tondeur, W., Tatchell, R. J. & Sanga, H. J. N. *Tropenmed. Parasit.* **28**, 499-506 (1977).
62. Purnell, R. E. *Adv. Parasit.* **15**, 83-132 (1977).
63. Gill, B. S., Bhattacharyulu, Y. & Kaur, D. *Res. vet. Sci.* **21**, 146-149 (1976).
64. James, D. M. *Int. J. Parasit.* **6**, 179-182 (1976).
65. Wilson, A. J., Paris, J., Luckins, A. G., Dar, F. K. & Gray, A. R. *Trop. Anim. Hlth Prod.* **8**, 1-12 (1976).
66. Preston, P. M. & Dumonde, D. C. *Clin. exp. Immun.* **23**, 126-138 (1976).
67. Schnur, L. F. *Ann. trop. Med. Parasit.* **70**, 371-373 (1976).
68. Farrell, J. P. *Expl Parasit.* **40**, 89-94 (1976).
69. Behnke, J. M. & Wakelin, D. J. *Helminth.* **51**, 167-175 (1977).
70. Cox, F. E. G. & Volter, A. *Ann. trop. Med. Parasit.* **60**, 297-303 (1966).
71. Cox, F. E. G. *Parasitology* **65**, 389-398 (1972).
72. Nelson, G. S., Amin, M. A., Saoud, M. F. A. & Teesdale, C. *Bull. Wld Hlth Org.* **38**, 9-17 (1968).
73. Massoud, J. & Nelson, G. S. *Bull. Wld Hlth Org.* **47**, 591-600 (1972).
74. Berezheko, V. K. & Nisenbaum, A. I. in *Antropozoogel'mintozy i Perspektivy ikh Likvidatsii* 7-8 (Moscow, 1975).
75. Nisenbaum, I. A. *Byulleten Vsesoyuznogo Instituta Gel'mintologii im K.I. Skryabina* **14**, 51-55 (1974).
76. Lainson, R. & Shaw, J. J. *J. trop. Med. Hyg.* **80**, 29-33 (1977).
77. Clark, I. A., Allison, A. C. & Cox, F. E. G. *Nature* **259**, 309-311 (1976).
78. Clark, I. A., Wills, E. J., Richmond, J. E. & Allison, A. C. *Infection Immun.* **17**, 430-438 (1977).
79. Ortiz-Ortiz, L., Gonzalez-Mendoza, A. & Lamayi, E. *J. Immun.* **114**, 1424-1425 (1975).
80. Holl, R. J. *exp. Med.* **142**, 299-311 (1975).
81. Kuhn, R. E., Vaughn, R. T. & Herbst, G. A. *Int. J. Parasit.* **5**, 557-560 (1975).
82. Weintraub, J. & Weinbaum, F. I. *J. Immun.* **118**, 2288-2290 (1977).
83. Smirnovski, L. L. & Larson, C. L. *Infection Immun.* **16**, 249-257 (1977).
84. Rau, M. E. & Tanner, C. E. *Nature* **256**, 318-319 (1975).
85. Thompson, R. C. A. *Vet. Rec.* **99**, 237 (1976).
86. Fauve, R. M. & Dedin, A. C. *r. hebdo. Seanc. Acad. Sci. D* **282**, 131 (1976).
87. Clark, I. A., Cox, F. E. G. & Alison, A. C. *Parasitology* **74**, 9-18 (1977).
88. Kierszenbaum, F. *Infection Immun.* **12**, 1227-1229 (1975).
89. Brener, Z. & Cardoso, J. E. *J. Parasit.* **62**, 645-646 (1976).
90. Schwartzberg, J. E., Krahelbuhl, J. L. & Remington, J. S. *Infection Immun.* **12**, 1037-1043 (1975).
91. Brocklesby, D. W. & Purnell, R. E. *Nature* **265**, 343 (1977).

Chemotherapy of parasitic infections

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Although several good antiparasitic agents are available, new drugs are needed for the treatment of diseases such as chloroquine-resistant malaria, Chagas' disease, leishmaniasis and filariasis. The 'semirational approach' should be the basis for their synthesis.

In 1975 the World Health Organization published a document: 'Tropical Diseases Today—The Challenge and the Opportunity' in which the research areas covered by the new special programme for research and training in tropical diseases are discussed¹. Five of these diseases—malaria, schistosomiasis, filariasis, trypanosomiasis and leishmaniasis—are caused by protozoa or helminths; and the first three affect some 600 million people².

In Europe, North America and North Asia malaria is largely a disease of the past^{3,4}. But the development of resistance to insecticides and antimalarial drugs⁵, together with profound changes in the political and economic climate, have resulted in a serious resurgence of malaria in the subtropical and tropical areas.

Schistosomiasis affects more than 200 million people in approximately 70 countries in Asia, Africa, South America and the islands of the Caribbean^{5,6}. The geographical distribution is related to the incidence of the aquatic snails which are the intermediate hosts. The greatest increase in this disease can without doubt be attributed to irrigation; the introduction of perennial irrigation in lower Egypt, for example, increased the prevalence of schistosomiasis from 5 to 80% within a few years⁷.

Ecological changes have also been found to enhance onchocerciasis (river blindness). The vectors, simuliids or 'blackflies' prefer rapid running water as their habitat. Although man-made lakes above dams tend to flood *Simulium* breeding grounds, the spillways from these dams are ideal breeding sites^{8,9}. At least 20 million people, living in tropical Africa, Yemen, Mexico and Central and South America are infected

with the causative agent of river blindness, *Onchocerca volvulus*^{8,10}.

Dams also are potential man-made breeding sites for the mosquito vectors of *Wuchereria bancrofti* and *Brugia malayi* and may contribute to an increased spread and intensity of lymphatic filariasis¹⁰. At the moment at least 250 million people are infected¹⁰.

Trypanosomiasis of tropical Africa, sleeping sickness, has been and still is a plague responsible for illness and serious mortality¹¹. Thanks to eradication campaigns the incidence of sleeping sickness was brought generally under control in the late fifties¹². But the prevalence of the disease has started to increase again since the mid-1960s^{9,12}.

The American form of trypanosomiasis, Chagas' disease, is widely distributed in rural areas of most of the South and Central American countries¹². The association of the vector hosts (bugs) with poor conditions of life together with the establishment of the disease in a wild animal reservoir make control of Chagas' disease almost impossible when chemotherapy is not coupled with drastic economic and sanitary improvements.

Leishmaniasis remains one of the major communicable diseases in the tropical and subtropical regions of the world¹³. In terms of the annual incidence of new cases, Peters estimates that there are many more human cases of leishmaniasis in the world than African trypanosomiasis¹⁴.

A thousand million people are at risk of contracting one or more of the diseases touched on¹⁵. Other important infections are for example amoebiasis, toxoplasmosis, ancylostomiasis, ascariasis, strongyloidiasis, trichinosis, trichuriasis, cysticercosis and hydatidosis. Several of these diseases are not the 'privilege' of people living in the tropics. For example, in many low-income rural communities in the South-east USA half or more of the children are infected with *Ascaris lumbricoides*.

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coides or *Trichuris trichiura* or both¹⁶. Warren estimated that four million inhabitants of the USA are infected with *Ascaris*—that is, about one-tenth of the persons infected with the lowly pinworm¹⁷.

Heavy *Ascaris* infections in the tropics have been associated with severe malnutrition and avitaminoses. Nutrition together with chemotherapy will alter both the incidence and the effects of parasitic diseases. In general, health programmes should form part of a broad programme for socioeconomic improvement¹⁹.

There is an expanding list of drugs active against a great number of parasitic diseases. Some of them will be described in the following paragraphs. The selection is partly based on the list of essential drugs published recently by WHO¹⁸. The properties of some newer drugs that either are available or will become available in the near future will be highlighted.

Antimalarial drugs

The antimalarial drugs selected for the WHO list¹⁸ are chloroquine, primaquine, pyrimethamine and quinine. For organisms resistant to the proposed drugs amodiaquine and sulfadoxine are indicated. The properties of these drugs have been reviewed many times^{12,19,20} and are too well known to warrant discussion here. However, it is of interest to note that the alkaloid quinine remains a venerable but vigorous participant in the emergency treatment of severe malaria¹⁹.

The emergence of chloroquine resistance in *Plasmodium falciparum*, the most pernicious plasmodial species for man, together with the well known characteristic toxic symptoms, cinchonism, caused by quinine, has resulted in an intensive search for new drugs. In the last decade more than 250,000 compounds have passed through a US Army search programme²¹. This has led to the development of mefloquine, a quinoline derivative without photosensitising properties and with good activity against chloroquine-resistant *falciparum* malaria²². However, mefloquine is still not available for general practice.

Hall *et al.*²³ recently found that a short course of quinine followed by a single dose of pyrimethamine-sulfadoxine (Fansidar) cured 92% of patients infected with *falciparum* malaria in an area of known chloroquine resistance.

While progress is being made much more research is needed. Of great help will be the continuous cultivation of *P. falciparum* in human erythrocytes which was recently accomplished by Trager and collaborators²⁴. This technique may contribute to biochemical and immunological studies and it provides a convenient new method for testing substances for antimalarial activity.

Antitrypanosomal drugs

Three antitrypanosomal drugs are listed by the WHO as essential for the treatment of sleeping sickness caused by African trypanosomiasis. They are melarsoprol (Mel B), pentamidine (Lomidine) and suramin (Antrypol, Germanin)¹⁸. The advantages and disadvantages^{19,25} of these compounds, the biochemical basis of the anti-trypanosomal activity and the value of the trypanocides as biochemical probes^{25,26} have been thoroughly discussed in many reviews.

A compound of considerable value for the treatment of human sleeping sickness is diminazene aceturate (berenil)²⁷. The problem is that berenil is marketed for veterinary use only. It is noteworthy that in veterinary medicine this drug is at present the only effective trypanocide left for use in cattle²⁵. In fact, a widespread development of resistance to quinapyramine (antrypol) and homidium bromide (ethidium bromide) has been found²⁵.

Two other drugs, levofuraltadone and nifurtimox (Lampit) have been tested in a limited number of clinical trials^{27,28}. Both might be welcome when no other alternative is available as in melarsoprol resistance²⁸.

Bloodstream forms of African trypanosomes are completely dependent on glycolysis for their energy supply. Under aerobic conditions NADH produced in glycolysis is re-oxidised in an indirect manner by the coupled action of NAD⁺-linked glycerol-3-phosphate dehydrogenase and a glycerol-3-phosphate oxidase (GPO) system²⁹. The GPO-system is blocked by salicylhydroxamid acid (SHAM) and is inoperative anaerobically²⁹. However, *in vitro* and *in vivo* SHAM did not drastically affect the viability of trypanosomes²⁹. Therefore, there must be an alternative pathway allowing ATP synthesis when GPO is blocked.

If, notwithstanding uncertainties concerning the pathway of glycolysis, aerobic (GPO system) and anaerobic glycolysis form the basis of trypanosomal energy production, simultaneous inhibition should result in cell death.

Glycerol inhibits, under anaerobic conditions, glucose utilisation by bloodstream trypanosomes³⁰. SHAM together with glycerol causes *in vitro* and *in vivo* loss of motility of *Trypanosoma brucei*³¹. However, the recurrence of parasitaemia always takes place 6 days after the treatment of rats. Therefore, other inhibitors of anaerobic glycolysis and of GPO should be tested. Recently, Bowman and Fairlamb³² found that suramin is a competitive inhibitor of GPO. Combination studies with suramin and inhibitors of anaerobic glycolysis might be of interest.

The lack of an active and specific drug against *Trypanosoma cruzi* is, as pointed out by Lumsden³³, one of the best known facts about Chagas' disease. The only compound mentioned in the WHO list¹⁸ is a nitrofurilidone derivative, nifurtimox (Lampit). There is no doubt that this drug is effective in the acute stage of the disease³⁴. A disadvantage is that patients have to be treated for 3 to 4 months³².

Another nitrofur derivative, SQ18 506 has been found more effective than nifurtimox against *T. cruzi* in laboratory animals³⁵. There are indications that SQ18 506 (trans-5-amino-3-[2-(5-nitro-2-furyl)vinyl]-1,2,3-oxadiazole) acts by reacting with DNA of *T. cruzi*³⁶.

The drug of choice in the treatment of amoebiasis, giardiasis and *Trichomonas vaginalis* infections³⁰, metronidazole (a 5-nitroimidazole), has also been found to have a suppressive action on *T. cruzi* in mice³⁴. Clinical trials in patients with acute infection showed benzimidazole, a 2-nitroimidazole, to be an effective and safe chemotherapeutic agent^{37,38}. Encouraging results were obtained in the treatment of chronic Chagas' disease^{38,39}.

An interesting approach to the treatment of Chagas' disease is to couple anti-protozoal drugs to a macromolecular carrier. This complex is taken up by way of phagocytosis⁴⁰. The phagosome subsequently fuses with a lysosome containing hydrolytic enzymes. These hydrolyse the carrier-drug complex liberating the drug inside the cell. Promising results with this lysosomotropic drug approach have been obtained with DNA-ethidium bromide complexes in mice infected with *T. cruzi*⁴⁰.

Antileishmanial agents

The two main groups of chemicals recommended for treatment of leishmaniasis are the pentavalent antimonials and the aromatic diamidines. Sodium stibogluconate is among the more potent antimonial drugs used in the treatment of leishmaniasis^{13,41}. This antimonial is well tolerated and gives prompt clinical and parasitological responses. Regardless of the considerable value of antimonial compounds in the treatment of leishmanial infections, response may be variable depending on the strains of the parasites¹³.

Pentamidine has become the diamidine of choice for treatment of antimony-resistant leishmaniasis¹⁹. Side effects seem in particular to be disturbances of blood sugar levels¹³. Kidney damage may also accompany its use⁴². Interestingly, although this drug shows some activity against *Leishmania mexicana* in tissue culture and is active against different types of leishmaniasis in man, it is ineffective at tolerated doses against

L. donovani and *L. tropica* in mice⁴³. This illustrates the drawbacks of animal models for the evaluation of new agents and indicates that screening and pharmacokinetic studies of drugs have to be done in different models. Recently, new models have been proposed, for example the tissue culture screen of Mattock and Peters⁴³ and the *in vivo* procedures using mice⁴³, hamsters⁴⁴, guinea pigs⁴¹. The hope is that the available models will lead to the development of less toxic antileishmanial compounds whose use will not be limited to uncomplicated cases but will also treat East-African visceral and South-American mucocutaneous leishmaniasis.

Antischistosomal drugs

Six decades ago tartar emetic (antimony tartrate) was introduced as a drug against *Schistosoma haematobium* infection in man⁴⁵. Despite the efforts ever since in the search for more effective schistosomicides, few drugs can be considered as antischistosomal agents of proven value.

Stibocaptate (antimony dimercaptosuccinate, Astiban), one of the more than 10,000 antimonials that have been synthesised⁴⁶, is still on the WHO list as a complementary drug¹⁸. Another, sodium antimony subgallate (Sb 273), is one of the main antischistosomal drugs used in China today⁴⁷. However, the duration of the treatment and the toxic effects observed make it unlikely that any of the antimonial compounds will procure a drug to control the disease by mass chemotherapy⁵.

The development of niridazole has provided an important new therapy for schistosomiasis due to *Schistosoma haematobium* (for reviews see refs 5, 6, 20). Side effects are generally mild. However, in a substantial percentage of patients serious reactions associated with the central nervous system have been noted. These side effects continue to preclude mass treatment with niridazole⁴⁸.

Niridazole has been found to inhibit glucose uptake⁴⁹ and to reduce glycogen stores⁵⁰. This glycogen depleting effect in schistosomes has been attributed to an inhibitory effect on the glycogen phosphorylase phosphatase⁵⁰. Niridazole is extensively metabolised in host liver⁵¹ and schistosomes⁵². It therefore has been suggested that a metabolite of niridazole might be the active principle.

Niridazole has been found to suppress profoundly cell-mediated hypersensitivity⁵³. A single oral dose of 1 mg per kg (1/800 the therapeutic dose for schistosomiasis), given to mice before the i.v. injection of *S. mansoni* eggs, suppressed granuloma formation around the eggs for periods up to 32 days. The predominant immunopathological reaction in schistosomiasis seems to be the granuloma formation induced and elicited by schistosome eggs. Control of the host reaction to eggs may therefore be a factor in the success of treatment⁵³.

In 1965 Rosi *et al.*⁵⁴ described the synthesis of hycanthone (Hyc) from lucanthone by *Aspergillus sclerotiorum*. The properties of this thioxanthone derivative with good activity against *S. haematobium* and *S. mansoni* infections have been fully discussed in different reviews^{5,6}.

Hyc has strong binding properties which attach it to acetylcholine (ACh) sites⁵⁵. The binding to the ACh receptor is much less easily reversed in *S. mansoni* than in *S. japonicum*⁵⁶. This may correlate with the differences in Hyc sensitivity of the 2 species. Hyc also causes degeneration of the tegument^{55,56} and inhibits noncompetitively the acetylcholinesterase activity of *S. mansoni*⁵⁵. The effectiveness of such inhibition is species related. It is very weak against bovine or eel esterase⁵⁵.

Although the mechanism of the therapeutic action of Hyc is still not fully understood, the differences between parasite and host-enzyme may be exploited in the search for new schistosomicides.

The organophosphorus cholinesterase inhibitor, metrifonate, is effective in the treatment of *S. haematobium* but lacks activity in animals and humans infected with *S. mansoni*⁵. These species differences cannot be accounted for by differences in the susceptibility of their cholinesterases to the drug⁵⁷. Much

more information is needed about the pharmacokinetics and the molecular basis of the species specificity of the drugs discussed so far.

Oxamniquine is a promising single-dose agent for the treatment of *S. mansoni* infections in rodents, primates and man⁶. No convincing activity was demonstrated against *S. japonicum* in mice, hamsters and primates or against *S. haematobium* in either host tested including man⁸. Initially this hydroxytetrahydroquinoline derivative was tested as an intramuscular preparation. Recently oral preparations (capsules) have been used with success^{8,58}. Although further studies are needed, oxamniquine seems to be an effective schistosomicidal oral drug suitable for use in endemic areas⁵⁸.

Praziquantel is a novel broad spectrum antischistosomal and anticestodal compound developed by joint research of Bayer and E. Merck^{59,60}. This pyrazinoisoquinolinone is active against sexually mature *S. mansoni*, *S. haematobium* and *S. japonicum* in rodents and monkeys⁶¹. It is noteworthy that praziquantel is also active against *S. intercalatum* and *S. mattheei*⁶¹. Preliminary clinical studies done in Zambia, Brazil, Japan and the Philippines indicate that praziquantel is a well tolerated effective schistosomicide against *S. haematobium*, *S. mansoni* and *S. japonicum*. If this can be confirmed in further trials, population-based chemotherapy of *Schistosoma* may become a reality in the near future (A. Davis, personal communication).

Incubation of *S. mansoni* in the presence of praziquantel results in a rapid and marked spastic paralysis⁶². This may be attributed to an effect on the membrane potential of the muscle cells of the schistosome (J. Bennett, personal communication). In sub-mitochondrial preparations praziquantel also inhibits various NADH-oxidising enzymes including the NADH-fumarate reductase⁶³. Further studies are needed to relate the effects upon electron transport systems and musculature.

Studies of Striebel⁶⁴ have shown that 4-isothiocyanato-4'-nitrodiphenylamine (C 9333-GO/CGP 4540) has antischistosomal activity when administered as a single oral dose to animals experimentally infected with *S. mansoni*, *S. haematobium* or *S. japonicum*. Thus this isothiocyanate shares with praziquantel the ability also to affect *S. japonicum* in its animal host.

Unlike hycanthone and niridazole this antischistosomal compound seems to be devoid of any detectable mutagenic activity when tested in the absence or in the presence of liver microsome preparations with *Salmonella* strains TA 98 and TA 100⁶⁵. However, the drug gives rise to a mutagenic metabolite in the urine of mice. Coadministration of succinyl-sulfathiazole with the drug decreases the formation of the mutagenic metabolite. This suggests a role of intestinal bacteria in the formation of the mutagenic compound⁶⁵.

The mode of action of this isothiocyanate is not known. Isothiocyanates have been described as possessing uncoupling properties⁶⁶. Therefore the interference with energy forming systems and with membranes should be investigated.

It is noteworthy that this compound has also been found to be active against intestinal nematodes, filariae and has been described as a new antihookworm compound^{64,67}.

In view of the rare, transient and mild side effects and the high degree of anthelmintic activity described in the studies cited here, this compound certainly warrants further research.

Other antischistosomal compounds in a relatively early stage of development are thiophene derivatives (for example, Ro 11-0761)⁶⁸ and benzodiazepines (for example, Ro 11-3128)⁶⁹.

Antinematodal drugs

Thirty years ago significant antifilarial activity was discovered in a series of piperazine derivatives⁷⁰. One of these compounds, diethylcarbamazine (DEC) still remains the only drug widely used for the chemotherapeutic control of lymphatic filariasis²⁰. DEC is effective against all stages of *Loa loa* and against the

microfilarial form of *Onchocerca volvulus*, the adult *Onchocerca* being insusceptible²⁰.

For onchocerciasis, suramin remains the only acceptable macrofilaricide⁷¹. According to Jaffe the clinical efficacy of suramin against this important filarial infection may be due in part to its ability to inhibit onchocercal dihydrofolate reductase⁷².

The toxicity of DEC and suramin in the treatment of onchocerciasis has recently been discussed by Anderson and Fuglsang⁷³. The slow drawn-out death of microfilariae caused by suramin is more damaging to the eye than the more rapid effects of DEC. The authors suggest that "it would seem rational to treat severe onchocerciasis with a course of DEC under steroid cover, in order to eliminate the microfilariae and then to kill the adult worms with a course of suramin"⁷³.

Levamisole has antifilarial activity in experimentally infected animals and several reports indicate that its activity against microfilariae of both *Wuchereria bancrofti* and *Brugia malayi* in some way resembles the activity of DEC^{20,74}. However, high and toxic doses are needed for micro- and macrofilaricidal activity for *O. volvulus*^{71,75}. More promising results were obtained by Prod'hon in using levamisole in combination with DEC against microfilariae of *O. volvulus*⁷⁶. Studies with such combinations certainly need more attention. Duke also suggests trials with high doses of mebendazole and its derivatives⁷¹.

Since its introduction in 1965, tetramisole and/or its levorotatory isomer levamisole is used in many countries against a broad range of nematodal infections in birds, pigs, ruminants and man⁷⁴. In terms of rapid action, tolerance and low dosage levamisole seems to be an excellent drug for the treatment of ascariasis in man²⁰. According to Gupta *et al.*⁷⁷ periodic deworming should form a part of the supplementary feeding programme if malnutrition is associated with ascariasis. Trimestrial administration of levamisole to heavily infected African school children has almost led to a complete eradication of *Ascaris*, whereas egg output of hookworms and output of *Strongyloides stercoralis* larvae was reduced by respectively 77 and 87% at the end of the year⁷⁸.

Tetramisole and levamisole affect *in vitro* the neuromuscular system of immature and adult nematodes⁷⁹. Pharmacological experiments seem to indicate that tetramisole acts by stimulation of ganglionic structures followed by a depolarising type of neuromuscular inhibition⁸⁰.

In vitro both anthelmintics also inhibit the fumarate reductase system in different parasitic helminths^{63,73}. Although there is evidence indicating that the effects of these drugs on the neuromuscular system are relevant for their mode of action it cannot be completely excluded that both effects together bring about the irreversible paralysis of the muscular system of some nematodes.

Two other important broad-spectrum anthelmintics, thiabendazole and cambendazole also inhibit the fumarate reductase.

The interference of both benzimidazole derivatives and the already discussed anthelmintics with this mitochondrial system may represent only a facet of their mode of chemotherapeutic action^{63,79}. This property may be of great help in elucidating the mitochondrial electron transport in parasitic helminths. Recent results of Kohler and Bachmann⁶³ indicate that the observed inhibition of NADH oxidase and NADH fumarate reductase reactions by these drugs may occur through interaction with the endogenous quinone function. A better knowledge of this part of the electron transport chain may perhaps lead to the development of a new, more potent inhibitor which may be a good lead chemical required for 'directed screening'.

A most important advance in the treatment of *Trichuris trichiura* has been obtained with mebendazole. This benzimidazole carbamate has been proved effective against *Ascaris*, *Enterobius*, hookworm, *Trichuris*, *Trichinella*^{20,81} and is now considered the anthelmintic of choice for the treatment of intestinal capillariasis due to *Capillaria philippinensis*⁸².

Studies on the effect of treatment *in vivo* with mebendazole or its fluorine analogue, flubendazole^{83,84} on the ultrastructural morphology of the encysted phase of *Trichinella spiralis* in rats have shown that the first morphological alterations in the infected area are seen in the larval surroundings. Similar results were obtained with trichlorfon (metrifonate) and thiabendazole⁸⁵. This shows that the disturbance of the host-parasite relationship may play a part in determining the anthelmintic activity of a compound.

Mebendazole affects the glucose uptake *in vitro* and *in vivo* and induces an increased utilisation of endogenous glycogen in nematodes and cestodes^{79,86,87}. Mebendazole also affects malate-induced phosphorylation in *Ascaris* mitochondria⁷⁹ and *in vitro* and *in vivo* it diminishes ATP synthesis and turnover of adenine nucleotides in *Moniezia expansa*^{86,87}. Progressive time-related micromorphological changes in cestodes and nematodes after treatment of the hosts with mebendazole have been reported^{88,89}. The cytoplasmic microtubules of the tegumental or intestinal cells of the parasites disappear in the first hours. This is followed by an accumulation of secretory vesicles in the Golgi areas, the cytoplasm becomes lytic and the absorptive cells degenerate completely. Similar results have been obtained with flubendazole.⁸⁸

The variety of effects observed renders it difficult to state which particular effect or sequence of effects is responsible for the eradication of worms. At present, therefore, the best approximation may be to conclude that mebendazole and other benzimidazole carbamates are capable of exerting a number of effects which all together account for their anthelmintic activity.

Anti-tapeworm drugs

In a recent review the properties of most of the old and novel anthelmintics against different cestodes have been summarised⁹⁰. Anticestodal drugs of special interest are still niclosamide (Yomesan) and bunamidine. The novel antischistosomal drug, praziquantel, proves to be effective in a single oral dose against mature cestodes in man and animals^{59,60}.

A number of benzimidazoles, for example, albendazole⁹¹, fenbendazole⁹², oxfendazole⁹³ and mebendazole⁹⁴ have been found to be effective against different tapeworms. It is noteworthy that mebendazole is active against the larval stages of *E. multilocularis* and *E. granulosus*. Based on animal experiments and on preliminary information^{95,96} mebendazole can be considered as an adjunct to surgery in humans suffering from hydatidosis.

Reflections

The number of novel products discussed in this review is somewhat contradictory to the WHO contention that "no major new drugs for the treatment of any of the tropical diseases have been produced within the past three decades"⁷⁹. Good drugs are now available for some of the serious parasitic infections that plague a great part of mankind, especially that part living in tropical and subtropical regions. Considering that an important aspect in the control of a great number of parasitic diseases is population-based chemotherapy or mass treatment, well-designed large-scale clinical trials should still be done, with most of the drugs or with combinations of them, in order to define the optimal dosage régimes. The WHO can help by providing resources for these field clinical trials.

However, several therapeutic drugs should be given only under appropriate medical supervision, since they may induce serious side effects especially in heavily infected patients. Such drugs should be replaced by better, safer remedies. There is increasing concern about mutagenicity and carcinogenicity of nitroheterocycles. A need therefore exists for new chemical leads to provide alternative compounds. Furthermore, new drugs should be made available, or existing drugs should be better evaluated, for the treatment of parasites resistant to some drugs.

There are several ways to look for new drugs. The last decade has seen some particularly rapid developments in the use of, for example, stereochemical and physicochemical parameters which, in conjunction with regression analyses, have made quantitative structure-activity analysis (QSAR) more capable of directing synthetic effort. However, in view of the complexity of the factors that affect the activity *in vivo* of a drug, the lack of knowledge concerning the mode of action of the lead compound and the almost complete ignorance of the host-parasite relationships, it is not surprising that we are unable to predict all the effects of a compound on the host and parasite. Nevertheless this 'rational approach' can be of help in programmes in which the principal goal is the improvement of the biological profile of a lead compound. The design of such a lead compound is a bigger problem. Even an adequate knowledge of the target will not permit one to predict with certainty that, to paraphrase Ehrlich⁸⁸, the new drug will fly in search of the enemy after the manner of bewitched balls.

Most of the available antiparasitic agents and, so far as I know, all the lead chemicals had their origin in a rational exploitation of massive empirical screening.

Obviously the best approach will be the so-called 'semi-rational approach' in which knowledge, gathered from the study of, for example, pharmacokinetics and mode of action of empirically discovered drugs, will be used as the basis for the synthesis of new drugs.

The research-based pharmaceutical industry is the entity best equipped for the search of new chemotherapeutic agents. The International Federation of Pharmaceutical Manufacturers Associations (IFPMA)⁹⁹ believes that "the industry's participation in the WHO Special Programme would be a major asset in guaranteeing its success". However, IFPMA also stated that "it is not sufficiently appreciated that the industry's formidable research and development costs must be paid for from current sales". Efforts should therefore be made by governmental and non-governmental authorities to provide better conditions in the developing world for the utilisation of new drugs and of existing effective drugs which go often totally or partially unused.

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1. *Tropical Diseases Today—The Challenge and the Opportunity* (World Health Organization, Geneva, 1975).
2. Rowe, D. S. *Santé du Monde*, 18–23 (1976).
3. Maugh, T. H. II. *Science* **196**, 413–416 (1977).
4. Noguer, A., Wernsdorfer, W., Kouznetsov, R. & Hempel, J. *WHO Chronicle* **32**, 9–17 (1978).
5. Werbel, L. M. in *Topics in Medicinal Chemistry* (eds Rubinowitz, J. L. & Meyerson, R. M.) **3**, 125–169 (Wiley, New York, 1970).
6. Katz, N. *Adv. Pharmac. Chemother.* **14**, 1–70 (1977).
7. Deom, J. in *Man Made Lakes and Human Health* (eds Stanley, N. F. & Alpers, M. P.) 387–400 (Academic, New York, 1975).
8. *WHO Tech. Rept. ser.* No. 597 (1976).
9. *Health—Sector Policy Paper* (World Bank, Washington, DC, 1975).
10. *WHO Tech. Rep. ser.* No. 542 (1974).
11. Janssens, P. G. *Ann. Soc. belge Méd. trop.* **57**, 191–200 (1977).
12. Gentilini, M., Duflot, B., Lagardère, B. & Danis, M. *Médecine Tropicale* **51–52** (Flammarion Médecine-Sciences, Paris, 1977).
13. Steck, E. A. *Prog. Drug Res.* **18**, 289–351 (1974).
14. Peters, W. in *Biochemistry of Parasites and Host-Parasite Relationships* (ed. Van den Bossche, H.) 523–535 (North-Holland, Amsterdam, 1976).
15. *WHO at the Crossroads. Report of the Director-General to the 30th World Health Assembly on the Work of WHO in 1976. WHO Chronicle* **31**, 207–238 (1977).
16. Blumenthal, D. S. *New Engl. J. Med.* **297**, 1437–1439 (1977).
17. Warren, K. S. *Am. J. trop. Med. Hyg.* **23**, 723–730 (1974).
18. *WHO Tech. Rep. ser.* No. 615 (1977).
19. Steck, E. A. *The Chemotherapy of Protozoan Diseases* (US Government Printing Office, Washington, DC, 1972).
20. Miller, M. J. *Prog. Drug Res.* **20**, 433–461 (1976).
21. Peters, W. *New Engl. J. Med.* **297**, 1261–1264 (1977).
22. Trenholme, G. M. *et al. Science* **190**, 792–794 (1975).
23. Hall, A. P. *Br. med. J.* (i), 1626–1628 (1977).
24. Trager, W. in *Biochemistry of Parasites and Host-Parasite Relationships* (ed. Van den Bossche, H.) 427–434 (North-Holland, Amsterdam, 1976).
25. Williamson, J. *Trop. Dis. Bull.* **73**, 531–542 (1976).
26. Newton, B. A. in *Biochemistry of Parasites and Host-Parasite Relationships* (ed. Van den Bossche, H.) 459–476 (North-Holland, Amsterdam, 1976).
27. Ruppel, J. F. & Burke, J. *Ann. Soc. belge Méd. trop.* **57**, 481–494 (1977).
28. Janssens, P. G. & De Muynck, A. *Ann. Soc. belge Méd. trop.* **57**, 475–479 (1977).
29. Oppenheer, F. R. *Energy-related Metabolism in Trypanosomes thesis*, Univ. of Amsterdam (1977).
30. Ryley, J. F. *Biochem. J.* **85**, 211–223 (1962).
31. Clarkson, A. B., Jr & Brohn, F. H. *Science* **194**, 204–206 (1976).
32. Bowman, I. B. R. & Fairlamb, A. H. in *Biochemistry of Parasites and Host-Parasite Relationships* (ed. Van den Bossche, H.) 501–507 (North-Holland, Amsterdam, 1976).
33. Lumsden, W. H. R. *Trans. R. Soc. trop. Med. Hyg.* **70**, 121–122 (1976).
34. Gutteridge, W. E. *Trop. Dis. Bull.* **73**, 699–705 (1976).
35. Gutteridge, W. E. *Trans. R. Soc. trop. Med. Hyg.* **68**, 160–161 (1974).
36. Sims, P. & Gutteridge, W. E. in *Biochemistry of Parasites and Host-Parasite Relationships* (ed. Van den Bossche, H.) 485–491 (North-Holland, Amsterdam, 1976).
37. Barclay, C. A., Cerisola, J. A., Lugones, H. & Ledesma, O. *10th Int. Congress of Chemotherapy. Abstr.* No. 116, Zurich, Switzerland, September 18–23 (1977).
38. Coura, J. R. & Ferreira, I. *10th Int. Congress of Chemotherapy. Abstr.* No. 118, Zurich, Switzerland, September 18–23 (1977).
39. Cerisola, J. A., Barclay, C. A., López Silva, J. & Mouzo, G. *10th Int. Congress of Chemotherapy. Abstr.* No. 117, Zurich, Switzerland, September 18–23 (1977).
40. Trouet, A., Jadin, J.-M. & Van Hoof, F. in *Biochemistry of Parasites and Host-Parasite Relationships* (ed. Van den Bossche, H.) 519–522 (North-Holland, Amsterdam, 1976).
41. Neal, R. A. & Miles, R. A. *Ann. trop. Med. Parasit.* **71**, 21–27 (1977).
42. Limbos, P., Thomas, H., Beelaerts, W. & Van Caesbroeck, D. *Ann. Soc. belge Méd. trop.* **57**, 495–499 (1977).
43. Peters, W. in *Biochemistry of Parasites and Host-Parasite Relationships* (ed. Van den Bossche, H.) 523–535 (North-Holland, Amsterdam, 1976).
44. Hanson, W. L., Chapman, W. L. & Kinnamon, K. E. *Int. J. Parasit.* **7**, 443–447 (1977).
45. Christopherson, J. B. *Lancet* **ii**, 325–327 (1918).
46. Friedheim, E. A. H. in *Chemotherapy of Helminthiasis I*, (eds Cavier, R. & Hawking, F.) 129–144 (Pergamon, Oxford, 1973).
47. Report of the American Schistosomiasis Delegation to the People's Republic of China *Am. J. trop. Med. Hyg.* **26**, 427–457 (1977).
48. Fontanilles, F. *Ann. N.Y. Acad. Sci.* **160**, 811–820 (1969).
49. Magzoub, M. *Comp. gen. Pharmac.* **3**, 401–409 (1972).
50. Bueding, E. & Fisher, J. *molec. Pharmac.* **6**, 532–539 (1970).
51. Faigle, J. W. & Keberle, H. *Ann. N.Y. Acad. Sci.* **160**, 544–557 (1969).
52. Hess, R., Faigle, J. W. & Lambert, C. *Nature* **210**, 964 (1966).
53. Mahmoud, A. A. F., Mandel, M. A., Warren, K. S. & Webster, L. T., Jr *J. Immun.* **114**, 279–283 (1975).
54. Rosi, D. *et al. Nature* **208**, 1005–1006 (1965).
55. Senft, A. W. & Hillman, G. R. in *Biochemistry of Parasites and Host-Parasite Relationships* (ed. Van den Bossche, H.) 619–628 (North-Holland, Amsterdam, 1976).
56. Hillman, G. R., Gibler, W. B. & Anderson, J. B. *Am. J. trop. Med. Hyg.* **26**, 238–242 (1977).
57. Bueding, E., Liu, C. L. & Rogers, S. H. *Br. J. Pharmac.* **46**, 480–487 (1972).
58. Katz, N., Zicker, F. & Pereira, J. P. *Am. J. trop. Med. Hyg.* **26**, 234–237 (1977).
59. Thomas, H. & Andrews, P. *Pestic. Sci.* **8**, 556–560 (1977).
60. Thomas, H. & Gönner, R. *Res. vet. Sci.* **24**, 20–25 (1978).
61. Gönner, R. & Andrews, P. *Z. Parasitenkde* **52**, 129–150 (1977).
62. Fetterer, R., Pax, R. & Bennett, J. *Am. Soc. Parasit.* (Las Vegas, November, 1977).
63. Köhler, P. & Bachmann, R. *Molec. Pharmac.* **14**, 155–163 (1978).
64. Striebel, H. P. *Experientia* **32**, 457–458 (1976).
65. Bueding, E., Batzinger, R. & Petterson, G. *Experientia* **32**, 604–606 (1976).
66. Miko, M. & Chance, B. *Biochim. biophys. Acta* **396**, 165–174 (1975).
67. Doshi, J. C. *et al. Am. J. trop. Med. Hyg.* **26**, 636–639 (1977).
68. Stohler, H. R. *Abstracts of the Tagung deutschsprachiger tropenmedizinischer Gesellschaften*, 49 (Lindau, Bodensee, March 24–26, 1977).
69. Stohler, N. R. *10th Int. Congress of Chemotherapy Abstr.* No. 62 Zurich, Switzerland, September 18–23 (1977).
70. Hewitt, R. T., White, E., Wallace, W. S., Stewart, H. W., Kushner, S. & Subbarow, Y. *J. Lab. clin. Med.* **32**, 1304 (1947).
71. Duke, B. O. L. *Abstracts of the Tagung deutschsprachiger tropenmedizinischer Gesellschaften*, 39 (Lindau, Bodensee, March 24–26, 1977).
72. Jaffe, J. J. in *Comparative Biochemistry of Parasites* (ed. Van den Bossche, H.) 219–233 (Academic, New York, 1972).
73. Anderson, J. & Fuglsang, H. *Trop. Dis. Bull.* **74**, 257–272 (1977).
74. Janssens, P. A. *J. Prog. Drug Res.* **20**, 348–383 (1976).
75. Duke, B. O. L. *Trans. R. Soc. trop. Med. Hyg.* **69**, 287–288 (1975).
76. Prod'homme, J. & Moreau, J. P. *XVII^e Conférence Technique de l'OCCGE*, Bobo-Dioulasso, April 11–14 (1977).
77. Gupta, M. C., Arora, K. L., Mithal, S. & Tandon, B. N. *Lancet* **i**, 108–110 (1977).
78. Gatti, F., Krubwa, F., Vandepitte, J. & Thienpont, D. *Ann. Soc. belge Méd. trop.* **52**, 19–32 (1972).
79. Van den Bossche, H. in *Biochemistry of Parasites and Host-Parasite Relationships* (ed. Van den Bossche, H.) 553–572 (North-Holland, Amsterdam, 1976).
80. Van Nueten, J. M. in *Comparative Biochemistry of Parasites* (ed. Van den Bossche, H.) 101–115 (Academic, New York, 1972).
81. Thienpont, D., Vanparijs, O. F. & Vandesteene, R. in *Trichinellosis* (ed. Kim, C. W.) 515–527 (Intext Educational, New York, 1974).
82. Singson, C. N., Banzon, T. C. & Cross, J. H. *Am. J. trop. Med. Hyg.* **24**, 932–934 (1975).
83. De Nollin, S., Borgers, M., Vanparijs, O. & Van den Bossche, H. *Parasitology* **69**, 55–62 (1974).
84. De Nollin, S. & Borgers, M. *Abstr. 4th Intern. Conference on Trichinellosis* Poznan, Poland (1976).
85. Kozar, Z., Zorzycki, J., Seniuta, R. & Martynowicz, T. *Expl Parasit.* **21**, 173–185 (1967).
86. Rahman, M. S. & Bryant, C. *Int. J. Parasit.* **7**, 403–409 (1977).
87. Rahman, M. S., Cornish, R. A., Chevis, R. A. F. & Bryant, C. *N.Z. vet. J.* **25**, 79–83 (1977).
88. Borgers, M., De Nollin, S., Verheyen, A., De Brabander, M. & Thienpont, D. in *Microtubules and Microtubular Inhibitors* (eds Borgers, M. & de Brabander M.) 497–508 (North-Holland, Amsterdam, 1975).
89. Verheyen, A., Borgers, M., Vanparijs, O. F. & Thienpont, D. in *Biochemistry of Parasites and Host-Parasite Relationships* 605–618 (North-Holland, Amsterdam, 1976).
90. Van den Bossche, H. in *The Biology of the Tapeworm Hymenolepis diminuta* (ed. Arai, H. P.) (Academic, New York, in the press).
91. Theodorides, V. J., Gyurik, R. J., Kignsbury, W. D. & Parish, R. C. *Experientia* **32**, 702–703 (1976).
92. Duwel, D. *Pestic. Sci.* **8**, 550–555 (1977).
93. Averkin, E. A. *et al. J. med. Chem.* **18**, 1164–1166 (1975).
94. Eckert, J. & Pohlenz, J. *Tropenmed. Parasit.* **27**, 247–262 (1976).
95. Bekhti, A. *et al. Br. med. J.* **1047**–1051 (1977).
96. Lüdin, C. E., Gyr, K. & Karoussos, K. J. *Int. med. Res.* **5**, 367–368 (1977).
97. WHO document TDR/WP/76.5.
98. Ehrlich, P. *Lancet* **ii**, 445–451 (1913).
99. Memorandum of the International Federation of Pharmaceutical Manufacturers Associations (IFPMA): The WHO Special Programme for Research and Training in Tropical Diseases and the Role of the Pharmaceutical Industry, Zurich (1978).

articles

Origin of chlorine and bromine in the oceans

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Continuous fluxing at a decreasing rate of mantle Cl and Br by volcanism throughout the Earth's history can account for the mass of Cl and Br, and the Cl/Br ratio, of the oceans.

THE origin of volatile elements in the Earth's surface reservoir (defined here as oceans, atmosphere and sediments, including evaporites) is a central geochemical problem. Rubey¹ pointed out that volatiles such as H₂O, CO₂, Cl, F, Br, I, N, S, and Ar are present in the surface reservoir in far greater amounts than could be accounted for by the weathering process which produced the sedimentary rocks now on the Earth's surface. He concluded that the surface reservoir inventory of these 'excess volatiles' accumulated gradually with geological time by the escape of volatiles from intrusive and extrusive rocks more or less continuously rising from the deep interior of the Earth. The extent of submarine volcanism was unknown to him at the time. Recently, Anderson^{2,3} re-evaluated Rubey's hypothesis for Cl and S by calculating the outgassing rate of island arcs using data on the production rate of island arcs and the mass loss of Cl and S during volcanism (as estimated from the difference in Cl and S contents of glass inclusions in phenocrysts, and glassy island arc rocks). He concluded that the release of Cl and H₂O by island arc volcanism alone, at the estimated current rate, could have produced in the Earth's history 0.3–4 times the surface reservoir inventory of Cl and H₂O, consistent with Rubey's model for the origin of excess volatiles. Anderson also calculated the flux of Cl into the oceanic crust by multiplying the Cl content of oceanic crustal rocks by the crustal production rate at spreading centres. He left open the question of whether all the Cl degassed at subduction zones derived from this flux or from an independent juvenile mantle source present beneath the subduction zones.

We recalculate here the rate of Cl and Br transfer from the Earth's interior to the surface reservoir by assuming that essentially all Cl and Br entering the Earth's crust at spreading centres and hotspots (which have not been previously considered) are eventually released to surface reservoirs (Fig. 1). We also assume that during partial melting in the mantle, Cl and Br quantitatively enter the melt. We use the results of Unni and Schilling^{4,7}, and Unni⁸, and unpublished data on the Cl content of undegassed mid-ocean ridge basalts and lavas from the Iceland, Azores and Hawaii hotspots (Table 1). Our calculation indicates that release of Cl and Br by mid-ocean ridge and hotspot volcanic activity during geological time can account for one-third of the Cl now in the surface reservoir. A continuous degassing model can account for most or all of the remaining surface reservoir Cl and Br content if two or more of the following assumptions are invoked: (1) the rate of volcanism and transfer of Cl and Br from the mantle is proportional to radiogenic heat production; (2) trapped within the lithosphere is an amount of magma ~2–3 times that segregated as oceanic

crust, and the Br and Cl contents of this magma are released during subduction; (3) before 1,500 Myr BP (the approximate time at which the asthenosphere became significantly depleted in large ionic lithophile elements, LILE) the Cl and Br content of the mantle source of mid-ocean ridge basalts was 2–6 times the present value.

We first consider the amount of Cl present, and later the Br. We adopt a value of 4.55×10^{19} kg for the surface reservoir Cl content (Table 2). Cl stored in the continental crust amounts to 2.27×10^{18} kg, or only 5% of the Cl contained in the surface reservoir; it is, therefore, negligible for the discussion here. The input of Cl from the Earth's interior (mantle) into the surface reservoir takes place by volcanism at (1) mid-ocean ridges (MOR); (2) island arc or cordillera–trench systems; (3) oceanic islands (including those on mid-ocean ridges, such as Iceland) and (4) continental rifts and plateau basalts (Fig. 1). The term hotspot will comprise types (3) and (4). The efficiency with which these volcanic processes carry volatiles into the surface reservoirs is, of course, mostly unknown. However, we can estimate a maximum (or potential) rate from the rates of eruption and the volatile content of lavas before effervescence during eruption^{4–8} (Table 1).

Mid-ocean ridge and island arc volcanism

We first consider degassing associated with mid-ocean ridge and island-arc volcanism. Our key assumption is that all Cl (and Br) transferred from the mantle to the oceanic crust–lithosphere system at the ridge axis eventually enters the surface reservoir. This release may occur during initial eruption and consolidation of lavas at mid-ocean ridge, hotspots and island arcs, during lithosphere subduction and oceanic crust dehydration⁹, by hydrothermal leaching, and by weathering of mantle derived subaerial volcanic rocks (Fig. 1). We assume that Cl and Br released at island-arcs are derived from the subducting oceanic lithosphere alone. With these assumptions, the chlorine degassing rate from mid-ocean ridge volcanism is equal to the product of the mid-ocean ridge crustal production rate and the Cl content of undegassed mid-ocean ridge basalts. Possible incorporation of Cl (and Br) into the ageing lithosphere by hydrothermal circulation and rock weathering need not be considered, because Cl (and Br) acquired this way will be released after some 200 Myr at subduction zones (Fig. 1). Similarly, consideration of Cl frozen in island arc andesites or subsequently stored in the continental crust by accretion of older arcs can be neglected, as the Cl content of the crust is only 5% that of the surface reservoir (Table 2).

The rate of generation of oceanic crust comprising layers 2 and 3 is 4×10^{13} kg yr⁻¹ (ref. 9), and the chlorine content is 48 ± 24 p.p.m. (Table 1). The latter value represents the average Cl content of mostly glassy undegassed basalts erupted well below the critical 500 m water depth at which H₂O, Cl and Br would effervesce, and located along three normal ridge segments of the Mid-Atlantic Ridge^{4,7}. The calculation implicitly assumes that the original compositions of both layers 2

Table 1 Average Cl and Br content of normal mid-ocean ridge basalts and oceanic island lavas (subaerial and submerged)*

	Cl (p.p.m.)	Tholeiites Br (p.p.m.)	Cl/Br	Cl (p.p.m.)	Alkali lavas Br (p.p.m.)	Cl/Br	Cl (p.p.m.)	Nephelinites Br (p.p.m.)	Cl/Br	Depth range (m)
Normal Ridge Segments	48±24 (27)	0.17±0.18 (22)	303±103 (22)							710–1,383
Reykjanes Ridge 61–54° N	62±25 (12)	0.26±0.12 (9)	222±70 (9)							2,545–4,160
Mid-Atlantic Ridge 52–49° N	25±7 (7)	0.07±0.02 (6)	369±53 (6)							2,545–4,160
Mid-Atlantic Ridge 34–28° N	46±18 (8)	0.15±0.07 (7)	351±106 (7)							1,950–4,280
average of averages	44±15	0.16±0.08	314±65							
Oceanic Islands (Subaerial)	70±40 (39)	0.25±0.15 (36)	289±79 (36)	383±254 (22)	1.20±0.85 (22)	348±84 (22)	1,008±300 (4)	2.54±0.7 (4)	397±19 (4)	
Iceland W zone	62±32 (20)	0.21±0.12 (17)	316±82 (17)							
Iceland NE zone	81±52 (15)	0.30±0.18 (15)	260±55 (15)							
Hawaiian Islands	67±7 (4)	0.27±0.19 (4)	280±122 (4)	383±319 (12)	1.11±1.1 (12)	386±73 (12)	1,008±300 (4)	2.52±0.7 (4)	397±19 (4)	
Azores Islands				382±164 (10)	1.31±0.53 (10)	302±75 (10)				
average of averages	70±10	0.27±0.03	285±28	383±1	1.21±0.14	344±59				
Oceanic Islands (Submerged)†	312±198 (32)	0.77±0.39 (26)	364±84 (26)							75–633
Iceland Platform (63.3–62.6° N)	186±29 (11)	0.67±0.13 (10)	282±27 (10)							
Azores Platform (MAR 40–31.5° N transect)	412±223 (16)	0.91±0.53 (11)	427±54 (11)							1,100–2,850
Terceira flank (Azores)§	444 (1)	0.90 (1)	494 (1)							2,295
Terceira trough (Azores)	461 (1)	1.05 (1)	441 (1)							2,600
Hawaii, Kilauea flank	145±26 (3)	0.44±0.11 (3)	334±27 (3)							1,400–4,680
average of averages	257±159‡	0.69±0.26	357±88							

Nos. in parentheses show no. of samples included. Underlined values are those used for Cl and Br input estimates in text.

* Data taken from refs 7, 8 and unpublished.

† Used for Cl content of undegassed lavas.

‡ Average computed from three areas; Hawaii, Iceland and an average of the three regions over the Azores Platform (439±25).

§ Could be considered as an alkali basalt (48.62% ol, 0.02% hyp, 51.35% Di).

and 3, before cooling and hydrothermal leaching or contamination, are tholeiitic and identical. The difference in the seismic velocities of layers 2 and 3 is thus assumed to be primarily due to mineral grain size caused by different rates of cooling. Any compositional differences are assumed to reflect secondary processes related to the ageing of the oceanic crust. The corresponding rate of Cl transferred from the mantle to the oceanic crust, and, if our assumptions are correct, to the surface reservoir, is $1.92 \times 10^{19} \text{ kg yr}^{-1}$ ($48 \times 10^{-6} \text{ kg Cl per kg rock} \times 4 \times 10^{13} \text{ kg yr}^{-1}$ of rock). At such a rate, $8.75 \times 10^{18} \text{ kg}$ of Cl would be released in 4,560 Myr—the age of the Earth—this is 19% of the surface reservoir Cl content of $4.55 \times 10^{19} \text{ kg}$. Our estimated Cl degassing rate is about half that of Anderson^{2,3} who used 150 p.p.m. for the average Cl content of new oceanic crust, and $2.8 \times 10^{13} \text{ kg yr}^{-1}$ for oceanic crust generation (Table 3).

Hotspot volcanism

Hotspots leave their imprint on the Earth's surface in three principal ways depending on where they are located^{10–12} (Holden and Vogt¹³ should be consulted for a more thorough appreciation of hotspot or mantle plume phenomena). First, they form chains of islands if present beneath the interiors of plates (such as the Hawaiian–Emperor Island Chain). Second, they occur as V-shaped aseismic ridges with the apex located on an active spreading plate margin. These aseismic ridges end at continental margin as plateau basalts which reflect the initial hotspot activity at the time of continental breakup and onset of drift¹¹ (for example, Faeroes–Iceland–East Greenland hotspot–aseismic plateau basalt system). Third, hotspots located beneath relatively stationary continental plates generate long persisting volcanic centres over unusually elevated continental regions (such as the Tibesti or Afar regions of Africa).

Hotspot lavas, like mid-ocean ridge volcanics, represent a flux of mantle-derived material to the Earth's surface. While their production rate is much less than that of crust at normal spreading centres, their Cl and Br contents are very high (Table 1), and this compels us to consider their contribution to global degassing (Fig. 1). Oceanic hotspots have a similar fate as the oceanic crust (Fig. 1). The Cl residence time (lifetime before destruction by erosion or subduction) in oceanic island hotspots is less than or equal to the 200 Myr residence time of Cl in the oceanic crust, because of additional wave action. The Cl residence time in the extrusive part of continental hotspot rock mass is also ~200 Myr (estimated assuming a thickness of 2 km

and an erosion rate of $10^{-8} \text{ km yr}^{-1}$ for temperate climates¹⁴). The Jurassic age of what remains of the Karoo and Parana plateau basalts, supports this estimate¹⁵. The Cl residence time in the intrusive part of continental hotspots may be longer, but we can still infer from the small mass of Cl stored in continental crust, that such Cl is eventually released to the surface reservoir. In view of these short Cl residence times, we assume that all hotspot rock Cl (and Br) is transferred to the surface reservoir either by effervescence during eruption^{4–8}, by weathering, or by hydrothermal activity and subduction of oceanic lithosphere including hotspots (Fig. 1). Thus we calculate the potential contribution of hotspot volcanism to Cl degassing as the product of the average undegassed Cl content of the hotspot rocks and the production rate of magmatic rocks at hotspots.

Estimating the average Cl content, and especially the production rate, is more complicated and less certain for hotspots than for mid-ocean ridges. The total volcanic production rate for the Iceland hotspot can be estimated from the crustal thickness and spreading rates, and is found to be $0.06\text{--}0.13 \text{ km}^3 \text{ yr}^{-1}$ ($8\text{--}16 \text{ km thick crust} \times 2 \times 10^{-5} \text{ km yr}^{-1}$ spreading rate $\times 400 \text{ km length}$). An independent estimate may be derived using the minimum plume fluxes estimated by Schilling (in preparation) from data on chemical and isotopic gradients found along the ridge axis on either side of such plumes, assuming 25% partial melting (Table 4). Minimum mantle plume fluxes estimated in this way for Iceland, the Azores, and the Galapagos are $0.41\text{--}0.82 \text{ km}^3 \text{ yr}^{-1}$, $0.9\text{--}1.8 \text{ km}^3 \text{ yr}^{-1}$, and $0.96 \text{ km}^3 \text{ yr}^{-1}$, respectively. Volcanic production rates estimated from these results (that is, the product of the mantle plume flux and the degree of partial melting assumed to be 25% by weight) together with our other estimate for Iceland and estimates of other workers for various hotspots, are summarised in Table 4. Based on these estimates we adopt a probable range of magma intrusion and extrusion rates of $0.01\text{--}0.2 \text{ km}^3 \text{ yr}^{-1}$ per hotspot.

Burke and Wilson¹² counted 122 intermittently active hotspots for the past 10 Myr. This corresponds to an annual lava production rate of $3.5 \times 10^{12} \text{ kg yr}^{-1}$, assuming a minimum rate of $0.01 \text{ km}^3 \text{ yr}^{-1}$ per hotspot. On the other hand, Morgan¹¹ recognised 20 hotspots continuously active in oceanic areas, and we can add at least 10 more for continental areas (Columbia River, North America; Patagonia, South America; Ferrar, Antarctica; Massif Central, France; Eiffel, Germany; Siberia; Syria-Arabia; Hoggar-Tibesti, Africa; Cameroon, Africa; and East Africa), for a total of 30 hotspots. This number would

Table 2 Amount of Cl and Br in various surface reservoirs

Reservoir	Mass (kg)	Concentration (p.p.m.)		Amount (kg)		Cl/Br
		Cl	Br	Cl	Br	
a Continental crust	1.08×10^{22} (49)	210 (52)	<1‡	2.27×10^{18}	$<1.08 \times 10^{16}$	300*
b Oceanic crust	4.8×10^{21} (49)	48	0.17	2.3×10^{17}	8.16×10^{15}	282
c Seawater	1.4×10^{21} (50)	19,353 (53)	67 (53)	2.71×10^{19}	9.38×10^{16}	289
d Sediments*	2×10^{21} (51)	9,200 (51)	5 (52)	1.84×10^{19}	1.0×10^{16}	1840
e Atmosphere†	5.1×10^{18} (50)	1.6×10^{-3} (54)	7.8×10^{-6} (54)	8.16×10^9	3.98×10^7	205
f Surface Res. c + d + e	3.4×10^{21}			4.55×10^{19}	1.04×10^{17}	438
g Earth	5.98×10^{24} (56)	25 (48)	0.134 (48)	1.50×10^{20}	8.01×10^{17}	188

Nos in parentheses indicate ref. to source of data; if not indicated, this work.

* Including evaporites.

† Excluding marine aerosols.

‡ Estimated on basis of Cl/Br ratio of ≈ 300 (3 p.p.m. given in ref. 52 and based on Behne⁵⁵, has been questioned⁴⁷).

correspond to $6\text{--}0.3 \text{ km}^3 \text{ yr}^{-1}$ of magma from hotspot activity. A minimum weighted average production rate of $1.5 \text{ km}^3 \text{ yr}^{-1}$ seems reasonable (six hotspots with the maximum rate: Iceland, Azores, Reunion-Deccan, Afar, Galapagos and Hawaii; and 30 with the minimum rate considered). This corresponds to $4.35 \times 10^{12} \text{ kg yr}^{-1}$ of rock, assuming an average density of $2.9 \times 10^3 \text{ kg m}^{-3}$. Both independent estimates of hotspot magma production rate are comparable, and represent about 10% of the oceanic crust generation rate we previously used.

Table 3 Estimated current input rates of Cl and Br to surface reservoir

Type	Rock mass (kg yr ⁻¹)	Cl (kg yr ⁻¹)	Br (kg yr ⁻¹)
Mid-ocean ridge volcanism	4×10^{13} (9)	1.92×10^9	6.8×10^6
Hotspot volcanism	2.4×10^{13} (2)	4.2×10^9 (2)	3.7×10^6
Island arc volcanism	4.35×10^{12}	1.29×10^9	3.7×10^6
	5×10^{12} (2)	3×10^9 (2)	5×10^7 (2)
Iceland*		1×10^7 (7, 8)	3.4×10^4 (7, 8)
Kilauea volcano, Hawaii*		2.8×10^7 (57)	

Nos in parentheses indicate ref. to source of data; if not indicated, this work.

* Fluxes of Cl and Br outgassing during subaerial eruptions.

An estimate of potential Cl transfer from the mantle by hotspot volcanism can be made from the average Cl content, before any possible degassing during eruption (Fig. 1), of the three major rock types—tholeiites, alkali basalts, and nephelinites—and their relative fluxes. This requires first estimating the fraction of Cl and Br lost by effervescence before or during eruption, which depends on the bulk composition and hydrostatic pressure of the melt^{6,7}. Iceland and Reykjanes basalts erupted subaerially or underwater at depths <500 m lose 90–40% of their original Cl and Br content, depending on the composition and the pressure at which they are quenched^{4,7}. By extrapolation, it was deduced^{6,7} that Cl and Br should cease to degas (presumably by effervescence during eruption and loss during subsequent cooling) from the lava when $\text{Na}_2\text{O}/\text{CaO}$ weight ratio in the melt reaches ~ 0.3 . Data for estimating the undegassed Cl and Br content of the various hotspot lava types are summarised in Table 1. We assume that alkali basalts and nephelinites, with $\text{Na}_2\text{O}/\text{CaO}$ ratios >0.3 , have retained nearly all of their original Cl and Br. Cl and Br concentrations for these rock types are taken as average values obtained for Hawaii and Azores alkali basalts (Table 1). On the other hand, due to degassing, Cl and Br concentrations of subaerial tholeiites from Hawaii and Iceland are respectively 54% and 67% lower than their submerged counterparts erupted below 500 m depth (Table 1). Averages of undegassed submerged tholeiites from Iceland, Azores and Hawaii are adopted for the Cl and Br

content of hotspot tholeiites (Table 1). The values used in our calculation are 257, 383 and 1,008 p.p.m. Cl for undegassed tholeiites, alkali basalts and nephelinites, respectively.

The volumetric proportions of these three lava types vary widely in time and space^{16–18}. Engel *et al.*¹⁹ suggested that the bulk of oceanic island edifices are composed of tholeiitic basalts, and lava from the alkali or nephelinite series are derivative melts capping only the summit of oceanic islands (such as Hawaii, Iceland, the Galapagos, and Reunion Islands^{20–22}). Most plateau basalts are also composed primarily of tholeiites²³. It also seems that tholeiitic hotspots erupt lava at the highest rate of the range previously estimated. On the other hand, exposed lavas from the linear chain of islands in French Polynesia in the Pacific^{24–26}, and in the Atlantic (the Canary Islands, Ascension, Tristan da Cunha, St Helena or Bouvet hotspots²⁷), tend to be dominated by alkali lavas, although their submerged base may be tholeiitic. Hotspots composed of alkali basalts seem to have erupted at the smallest rate of the range we estimated. Finally, some plateau basalts such as the Afar or the Mid-Atlantic Ridge transect over the Azores platform, produce dominantly alkali- and Cl-rich tholeiites with no normative nepheline^{28,29} (Table 1). Relative abundances of lava types for hotspot volcanism are not sufficiently well documented to make an accurate overall weighting possible. However, in general it seems that the Cl content of tholeiites, alkali basalts, and nephelinites are roughly inversely related to their flux rates. Nephelinites occur only in minor amounts compared to tholeiitic and alkali basalts and as a result contribute <10–20% of the Cl budget for hotspots. To be consistent with our weighting for the overall production rate of lava from hotspot activity, we will consider six hotspots with a production rate of $0.2 \text{ km}^3 \text{ yr}^{-1}$, made of 0.9

Table 4 Estimates of hotspot magma production rates*

Hot spot	Production rate (km ³ yr ⁻¹)	Ref.
Hawaiian Emperor Island Chain	0.012–0.018	58
Island of Hawaii	0.05	59
	0.11	60
Columbia Plateau	0.1	61
Iceland	0.23–0.45†	J.-G. S. unpublished
	0.026	62
	0.06–0.13‡	This work
Azores†	0.1–0.2	J.-G. S. unpublished
Galapagos†	0.24	J.-G. S. unpublished
Hoggar, Africa	0.008	63
Cameroon	0.012	63
East Africa	0.02	64
Ethiopia	0.008	65

* Extrusive, unless indicated.

† Includes extrusives and intrusives.

‡ Based on crustal thickness 8–16 km, spreading rate $2 \times 10^{-5} \text{ km yr}^{-1}$ and 400 km length (extrusive + intrusive).

tholeiites, 0.09 alkali basalts and 0.01 nephelinites, and a resulting weighted average of 276 p.p.m. of Cl; and 30 hotspots with a production rate of $0.01 \text{ km}^3 \text{ yr}^{-1}$ composed of 0.82 alkali basalts, 0.12 tholeiites and 0.03 nephelinites or a weighted average of 383 p.p.m. Cl (Table 1). The corresponding overall Cl production rate by hotspots is $1.29 \times 10^9 \text{ kg yr}^{-1}$ ($6 \times 0.2 \text{ km}^3 \text{ yr}^{-1} \times 2.9 \times 10^{12} \text{ kg km}^{-3} \text{ rock} \times 276 \times 10^{-6} \text{ kg Cl per kg rock} + 30 \times 0.01 \text{ km}^3 \text{ yr}^{-1} \times 2.9 \times 10^{12} \text{ kg km}^{-3} \text{ rock} \times 383 \times 10^{-6} \text{ kg Cl per kg rock}$). The result is close to our estimate of production rate for mid-ocean ridge volcanism ($1.92 \times 10^9 \text{ kg yr}^{-1}$ of Cl).

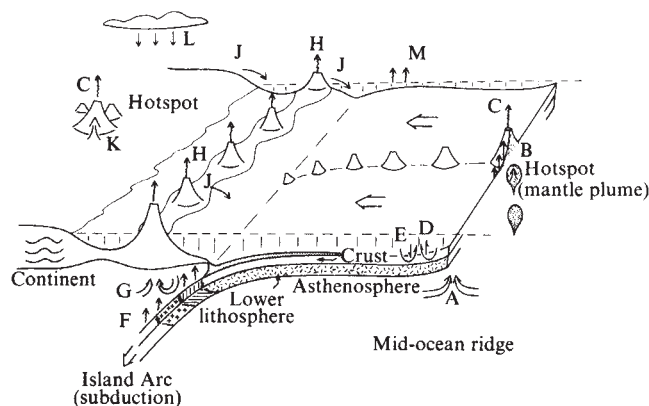


Fig. 1 Schematic diagram (not to scale) showing the geological cycle of chlorine (and bromine) associated with seafloor spreading and hotspot volcanism. Inputs of Cl into the surface reservoir (ocean, atmosphere and sediments) are the following: A, Cl transport from the asthenosphere (LILE-depleted) by mid-ocean ridge volcanism and temporarily stored in the oceanic crust; B, Cl transfer from the mantle (LILE-enriched) by hotspot or mantle plume derived volcanism (densely dotted blobs); C, Cl outgassing associated with subaerial hotspot volcanism (and mid-ocean ridge segments located above the 500 m critical depth of degassing); D, low temperature seawater–basalt exchange of Cl (weathering); E, hydrothermal circulation and metasomatic alteration of the oceanic crust; F, Cl outgassing of the ocean crust by dehydration and metamorphism during subduction of the lithosphere (crosses and hatched patterns represent distinct metamorphic facies); G, Cl transfer from the asthenosphere by island arc volcanism and temporarily stored in island-arc volcanics; H, Cl outgassing associated with island-arc volcanism; J, Cl transport by subaerial weathering; K, Cl transfer from the mantle (LILE-enriched) by hotspot volcanism on continents; L, washout of cyclic salts in rain; M, seaspray of salt. Light stippling, sediment layer 1. Crust layer includes layer 2 (basalt) and 3 (layered intrusives), but both layers are not shown for clarity. Random dashed pattern is for lower lithosphere (mantle residue after partial melt and melt segregation) which may include trapped gabbroic intrusives (not shown for clarity). Entire lithosphere includes crust and lower lithosphere. Small double arrows indicate probable convective flow patterns in the mantle. Large double arrows indicate horizontal direction of lithosphere plate motion due to seafloor spreading.

Cl and Br budget

The resulting annual mantle to surface reservoir rate of Cl transfer by mid-ocean ridge and hotspot volcanism is $3.21 \times 10^9 \text{ kg yr}^{-1}$. At such a rate, these two modes of volcanism would produce in 5,560 Myr $1.5 \times 10^{19} \text{ kg}$ of Cl, or 32% of the present sediment–seawater reservoir ($3.27 \times 10^9 \text{ kg yr}^{-1}$ of Cl $\times 4.56 \times 10^9 \text{ yr}$).

Our estimated rate of Cl transfer from the mantle to the surface reservoir probably represents a minimum for the following reasons.

First, it is reasonable to assume that during geological time the rate of crustal production has been directly proportional to the rate of heat production from natural radioactivity within the Earth³⁰. In this case, the ratio ($R_{0-4.56}$) of the mass of crust produced during the age of the Earth to the mass which would

have been produced if the production rate had always been equal to the present value, is given by the relationship:

$$R_{0-4.56} = \frac{\int_0^T \sum Q_i(t) dt}{\int_0^T \sum Q_i(0) dt} = \frac{\sum_i Q_i(0) [\exp(\lambda_i T) - 1] / \lambda_i}{T \sum_i Q_i(0)}$$

where $R_{t_1-t_2}$ = the ratio between ages t_1 and t_2 ; $Q_i(t)$ is the heat production rate of isotope i at time t (increasing towards the past); $Q_i(0)$ is the present heat production rate; T is the age of the Earth, 4,560 Myr; and λ_i = decay constant of species i . Using parameters listed in Tables 5 and 6, the ratio $R_{0-4.56}$ is 3.3, 2.6 and 2.0 for the chondritic Earth model, carbonaceous chondrite Earth model, and Wasserburg *et al.*³¹ preferable Earth model ($K/U = 10^4$), respectively (Tables 5 and 6). In other words, the average integrated transfer rate of Cl from the mantle to surface reservoir by volcanism throughout the Earth's history could reasonably have been about twice that of our previous estimate of $3.3 \times 10^9 \text{ kg yr}^{-1}$ for the present. On this basis alone, 65% of the Cl present in the surface reservoir could have been generated by volcanism during geological time.

Second, we may speculate that the oceanic crust represents only a fraction of the magma generated during lithosphere production. In this case, Cl contained in melts trapped within the lithosphere (that is, gabbroic intrusions on cooling) and subsequently released during subduction could increase our previous estimate. Indeed, Bottinga and Allegre³² estimate that 4–5 times more magma is produced than is evident from the 3-km combined thickness of layers 2 and 3A, or 2.4–3 times more than our estimate assuming a 5-km thick oceanic layer. Such magma entrapment is also implied by the fact that the ratio of the thickness of the oceanic crust to that of the lithosphere (taken as 75 km), is 0.067. This would correspond to 6.7% partial melting assuming complete melt segregation, whereas petrological considerations suggest that mid-ocean ridge basalts may be the product of some 25–30% partial melting of the asthenosphere³³. If the petrological arguments prevail we must conclude that 3 times as much magma is trapped within the lithosphere as emplaced as oceanic crust. In this case the potential Cl released to the surface reservoir by mid-ocean ridge and hotspot volcanism would be $4.1 \times 10^{19} \text{ kg}$ of Cl in 4,560 Myr, or 90% of the amount presently stored in the surface reservoir.

Third, the mantle source of mid-ocean ridge basalts may not have always been as depleted in Cl as it is now. Radiogenic isotope studies suggest that the asthenosphere source of mid-ocean ridge basalts was depleted in LIL elements, and by inference Cl, by partial melting episodes some 1,000–2,000 Myr BP (refs 34–38).

The Cl budget could be balanced if it were assumed that before 1,500 Myr BP, the Cl content was 6.3–1.8 times the present value, depending on whether volcanism occurred at a constant rate or at a rate proportional to heat production by natural radioactivity (for example, total Cl produced = $4.55 \times 10^{19} \text{ kg} = 4.56 \times 10^9 \text{ yr} \times R_{0-4.56} \times \text{hotspot production rate of Cl} + 1.5 \times 10^9 \text{ yr} \times R_{0-1.5} \times \text{modern MOR production rate of Cl} + (4.56 - 1.50) \times 10^9 \text{ yr} \times R_{1.5-4.56} \times E \times \text{MOR production rate of Cl}$; solving for E , using R values in Tables 5 and 6, gives $E = 1.8$, where E is the Cl content of MOR basalts older than 1,500 Myr, divided by that of modern MOR basalts, and where

$$R_{t_1-t_2} = \frac{\int_{t_1}^{t_2} \sum Q_i(t) dt}{\int_{t_1}^{t_2} \sum Q_i(0) dt}$$

A chlorine content at least 2–3 times higher before 1,500 Myr BP would not be unlikely by analogy with the distribution of light RE relative to heavy RE of mid-ocean basalts and Archean lavas³⁹. The $(\text{La/Sm})_{\text{EF}}$ (enrichment factor ratio relative to chondrites) of Archean basaltic komatiites is 1.5–2.5 higher than the value of modern mid-ocean ridge basalts³⁹. The

(La/Sm)_{EF} of nine Archean peridotitic komatiites reported by Sun and Nesbitt³⁹ average 0.78; whereas model estimates of the mantle source of modern mid-ocean ridge basalts are in the range 0.3–0.6 (ref. 40). Furthermore ¹⁴³Nd/¹⁴⁴Nd data indicate that the Nd/Sm of the Earth mantle before 1.5 Myr was chondritic^{36–38}, suggesting again an Archean mantle richer in incompatible elements. As Cl is likely to be at least as incompatible as La and Nd during partial melting in the mantle at high pressure, a similar enrichment of Cl in Archean relative to modern times could also be anticipated.

Using the same method, we estimate that the Br transferred from the mantle to surface reservoirs by mid-ocean ridge and hotspot volcanism is 1.05×10^7 kg yr⁻¹ of Br compared to 3.21×10^{19} kg yr⁻¹ for Cl. The corresponding current ratio of Cl to Br delivery is 306, which is remarkably close to the Cl/Br ratio of seawater (289), but is lower than the present surface reservoir value of 438 (Table 2). Possible causes for the discrepancy are discussed below.

Table 5 Parameters used for calculating values of *R* given in Table 6

	²³⁸ U	²³⁵ U	²³² Th	⁴⁰ K
$\lambda \times 10^{10}$ (yr ⁻¹)	1.537	9.722	0.4916	5.48 (66)
Radiogenic heat production ⁶⁷ (J yr ⁻¹ kg-isotope ⁻¹)	2.96×10^3	1.80×10^4	8.38×10^2	9.11×10^2

⁴⁰K/ Σ K = 1.8×10^{-4} atom ratio⁶⁸; ²³⁵U/²³⁸U = 1/137.8; age of Earth = 4,560 Myr (ref. 69). Nos in parentheses indicate ref. to source of data.

Table 6 Values of *R* calculated for materials with three different compositions*

Model	K/U	Th/U	<i>R</i> _{0–4.56}	<i>R</i> _{0–1.5}	<i>R</i> _{1.5–4.56}
Earth (31)	1.00×10^4	3.70	2.0	1.20	2.5
Chondrite (31)	7.86×10^4	3.70	3.3		
Orgueil (70–72)	2.86×10^4 †	3.12	2.6		

Nos in parentheses indicate ref. to source of data; if not indicated, this work.

* See text for definition of *R*. Also note that *R* depends only on elemental ratios and not on absolute abundances of U, Th and K.

† K is taken as the average of tabulated K concentrations for this meteorite (ref. 70, 160).

Conclusions

The above calculations indicate three remarkable similarities. First, the rate at which Cl is transferred from the mantle by mid-ocean ridge and hotspot volcanism is comparable to the rate at which Cl is outgassed at island arcs^{2,3,41}; this point lends credibility to our assumption that Cl and Br associated with lithosphere formation and hotspot activity are outgassed rather than recycled into the mantle (Fig. 1). Second, the geological time integrated rate of Cl and Br transfer from the mantle to the surface reservoir by volcanism is comparable to the masses of Cl and Br in the surface reservoir. Third, the ratio of Cl to Br in the surface reservoir is, considering the uncertainties, relatively close to the ratio at which, according to our calculations, these two volatiles are transferred to the surface reservoir. These observations are consistent with, but do not prove, the hypothesis that the surface reservoir Cl and Br content has accumulated throughout geological time by volcanic processes such as now occur, although the accumulation rate may have been higher in the past because of a less depleted mantle and higher heat production. Our calculations show that no episode of early outgassing need be invoked to explain the surface reservoir Cl and Br content.

At the same time, there are large uncertainties which prevent us from reaching any firm conclusions. First, our adopted surface reservoir Cl content value (Table 2), which does not

include the Mediterranean evaporites⁴² and evaporites associated with ocean openings^{43,44} may be an underestimate. The extent of evaporite deposits hidden under rifted continental margins^{43,44} and young oceans such as the Red Sea⁴⁵ remains uncertain. If the mass of newly discovered and undiscovered evaporite Cl equals the mass of evaporites of 1.28×10^{19} kg estimated by Garrels and MacKenzie¹⁴ (Table 2), the surface reservoir Cl content estimate would be raised by only 28%. This change would hardly influence our conclusions, but if the mass of recently discovered plus undiscovered evaporites were far greater, our conclusions would have to be revised. Second, we have noted that our estimate of the ratio of Cl to Br delivery (306) by current hotspots and mid-ocean ridges, although close to that of seawater (298), is lower than the present surface reservoir value of 438. This discrepancy may be real and reflect shortcomings in our assumptions and calculations. On the other hand, it may be due to an underestimate of the sedimentary reservoir Br content. Price *et al.*⁴⁶ have shown that the Br content of organic rich Barents Sea sediments range from 12 to 257 p.p.m., compared with the assumed weighted average of 5 p.p.m. for sediments in general (see Table 2). We believe that more data on the surface reservoir Br content are needed before we can draw any conclusions from comparing the current Cl/Br release rate and the surface reservoir Cl/Br ratio. Third, another uncertainty reflects questions about the fate of Cl in the lithosphere. We have assumed that it is quantitatively outgassed, but cannot eliminate the possibility that it is largely recycled into the mantle. If this were the case, it would be necessary to postulate another source for the flux of Cl released at island arcs as estimated by Anderson²³ and Cadle⁴¹—conceivably either mantle-derived juvenile Cl and Br, or seawater halogens contained in the pores of subducted sediments. We had hoped to use the Cl/Br ratio of seawater, degassed and undegassed basalts and volcanic gases to fingerprint the source of Cl and Br evolved at island arcs, and contained in the oceans, but the ratios are too similar and the uncertainties on weighted averages too great to reach definite conclusions. The Cl/Br ratios of submerged undegassed hotspot tholeiites, glassy LIL-depleted MOR tholeiites, degassed subaerial tholeiites, and undegassed hotspot alkaline and nephelinitic basalts range from 222 to 494 (Table 1); whereas the Cl/Br ratio of volcanic rocks and gases from the Japan island arc averages 333 ± 222 and 676 ± 142 , respectively⁴⁷, and that of seawater 298. Furthermore, the fact that oceanic and ocean island volcanic rocks have Cl/Br ratios similar to the seawater obviates the possibility of using the ratio to ascertain whether Cl and Br released at island arcs are derived from intrusive and extrusive rocks contained in subducted lithosphere, recycled seawater, or directly from a juvenile mantle source. Independent evidence is required to resolve this question. On the other hand, the Cl/Br ratio of volcanics listed above do indicate that factors and processes which influence the chemistry of hotspot and MOR basalts (degree of partial melting, extent of fractional crystallisation, degassing and volatilisation) do not greatly fractionate Cl relative to Br^{4–8}. Finally, we note that the amount of Cl stored in the surface reservoir plus crust (4.8×10^{19} kg of Cl, Table 2) represents about one-third of the total inventory for the Earth, assuming Ganapathy and Anders⁴⁸ average Cl content of 25 p.p.m. for the overall Earth. This suggests that the Earth interior has lost one-third of its original Cl content by outgassing.

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1. Rubey, W. W. *Geol. Soc. Am. Bull.* **62**, 1111–1148 (1951).
2. Anderson, A. T. *Geol. Soc. Am. Bull.* **85**, 1485–1492 (1974).
3. Anderson, A. T. *Rev. Geophys. Space Phys.* **13**, 37–55 (1975).
4. Unni, C. K. & Schilling, J.-G. *Trans. Am. geophys. Union* **55**, 456 (1974).
5. Unni, C. K. & Schilling, J.-G. *Trans. Am. geophys. Union* **56**, 469 (1975).
6. Unni, C. K. & Schilling, J.-G. *Trans. Am. geophys. Union* **58**, 525–526 (1977).

7. Unni, C. K. & Schilling, J.-G. *Nature* **272**, 19–23 (1978).
8. Unni, C. K. thesis, Univ. Rhode Island (1976).
9. Fyfe, W. S. & McBirney, A. R. *Am. J. Sci.* **275A**, 285–297 (1975).
10. Wilson, J. T. *Can. J. Phys.* **41**, 863–870 (1963).
11. Morgan, W. J. *Nature* **230**, 42–43 (1971).
12. Burke, K. & Wilson, J. T. *Scient. Am.* **235**, 46–57 (1976).
13. Holden, J. C. & Vogt, P. R. *Trans. Am. geophys. Union* **58**, 573–580 (1977).
14. Garrels, R. M. & MacKenzie, *Evolution of Sedimentary Rocks*, 397 (Norton, New York, 1971).
15. McDougall, I. J. *geophys. Res.* **68**, 1535–1545 (1963).
16. McBirney, A. R. & Gass, I. G. *Earth planet. Sci. Lett.* **2**, 265–276 (1967).
17. Aumento, F. *Earth planet. Sci. Lett.* **2**, 225–230 (1967).
18. McDougall, I. *Geol. Soc. Am. Bull.* **75**, 107–128 (1964).
19. Engel, A. E., Engel, C. G. & Havens, R. G. *Geol. Soc. Am. Bull.* **76**, 719–734 (1965).
20. Stearns, H. T. *Hawaii Div. Hydrog. Bull.* **8**, 106 (1946).
21. McBirney, A. R. & Williams, H. *Geol. Soc. Am. Mem.* **118**, 197 (1969).
22. Upton, B. G. J. & Wadsworth, W. J. *Phil. Trans. R. Soc. A271*, 105–130 (1972).
23. Turner, F. J. & Verhoogen, J. *Igneous and Metamorphic Petrology*, 694 (McGraw-Hill, New York, 1960).
24. Duncan, R. A. & McDougall, I. J. *volc. geothermal. Res.* **1**, 197–227 (1976).
25. McBirney, A. R. & Aoki, K.-I. *Geol. Soc. Am. Mem.* **116**, 523–556 (1968).
26. Carmichael, I. S. E., Turner, F. J. & Verhoogen, J. *Igneous Petrology*, 739 (McGraw-Hill, New York 1974).
27. Baker, P. E. in *The Ocean Basins and Margins, I* (eds Nairn, A. E. M. & Stehli, F. G.) 493–553 (Plenum, New York, 1973).
28. Barberi, F. & Varet, J. *Bull. Volcanol.* **34**, 848–917 (1970).
29. Schilling, J.-G. *Earth planet. Sci. Lett.* **25**, 103–115 (1975).
30. Dickinson, W. R. & Luth, W. C. *Science* **174**, 400–404 (1971).
31. Wasserburg, G. J., MacDonald, G. J. F., Hoyle, F. & Fowler, W. A. *Science* **143**, 465–468 (1964).
32. Bottinga, Y. & Allegre, C. *Phil. Trans. R. Soc.* (in the press).
33. Green, D. H. & Ringwood, A. E. *Contr. mineral. Petrol.* **15**, 103–190 (1967).
34. Tatsumoto, M. *Science* **153**, 1094–1101 (1966).
35. Gast, P. W. *Geochim. cosmochim. Acta* **32**, 1057–1086 (1968).
36. DePaolo, D. J. & Wasserburg, G. J. *Geophys. Res. Lett.* **3**, 249–252 (1976).
37. DePaolo, D. J. & Wasserburg, G. J. *Geophys. Res. Lett.* **3**, 743–746 (1976).
38. O'Nions, R. K., Hamilton, P. J. & Evensen, N. M. *Earth planet. Sci. Lett.* **34**, 13–22 (1977).
39. Sun, S.-S. & Nesbitt, R. W. *Contr. mineral. Petrol.* **65**, 301–325 (1978).
40. Schilling, J.-G. *J. geophys. Res.* **80**, 1459–1473 (1975).
41. Cadle, R. D. J. *geophys. Res.* **80**, 1650–1652 (1975).
42. Ryan, W. B. F. *et al. Init. Rep. DSDP* **13** (1973).
43. Bonatti, E., Ball, M. & Schubert, C. *Naturwissenschaften* **57**, 107–108 (1970).
44. Burke, K. *Geology* **3**, 613–616 (1975).
45. Whitmarsh, R. B. *et al. Init. Rep. DSDP* **23** (1974).
46. Price, N. B., Calvert, S. E. & Jones, P. G. W. *J. mar. Res.* **28**, 1–34 (1970).
47. Sugiura, T. *Bull. chem. Soc. Jap.* **41**, 1133–1139 (1968).
48. Ganapathy, R. & Anders, E. *Geochim. cosmochim. Acta, Suppl.* **5**, 1181–1206 (1974).
49. Gast, P. W. in *Nature of the Solid Earth* (ed. Robertson, E. C.) 19–40 (McGraw-Hill, New York, 1972).
50. Stacey, F. D. *Physics of the Earth* (Wiley, New York, 1969).
51. Garrels, R. M. & MacKenzie, F. T. *Mar. Chem.* **1**, 27–41 (1972).
52. Turekian, K. K. in *Encyclopedia of Science and Technology* 2nd ed, 627–630 (McGraw-Hill, New York, 1971).
53. Pytkowicz, R. M. & Kester, D. R. *Oceanogr. mar. Biol. A. Rev.* **9**, 11–60 (1971).
54. Rahn, K. A. *Tech. Rep.*, Univ. Rhode Island (1976).
55. Behne, W. *Geochim. cosmochim. Acta* **3**, 186–215 (1953).
56. Ringwood, A. E. *Composition and Petrology of the Earth's Mantle* (McGraw-Hill, New York, 1975).
57. Kuroda, P. K. & Sandell, E. B. *Geol. Soc. Am. Bull.* **64**, 879–896 (1953).
58. Bargar, K. E. & Jackson, E. D. *J. Res. U.S. Geol. Survey* **2**, 545–550 (1974).
59. Decker, R. W. *Trans. Am. geophys. Union* **52**, 371 (1971).
60. Swanson, D. A. *Science* **175**, 169–170 (1972).
61. Baksi, A. K. & Watkins, N. D. *Science* **180**, 493–496 (1973).
62. Jakobsson, S. P. *Lithos* **5**, 365–386 (1972).
63. Black, R. & Girod, M. in *African Magmatism and Tectonics* (eds Clifford, T. N. & Gass, I. G.) 185 (Oliver and Boyd, Edinburgh, 1970).
64. King, B. C. in *African Magmatism and Tectonics* (eds Clifford T. N. & Gass, I. G.) 263 (Oliver and Boyd, Edinburgh, 1970).
65. Gass, I. G. *Phil. Trans. R. Soc. A267*, 369–381 (1970).
66. Beckinsale, R. D. & Gale, N. H. *Earth planet. Sci. Lett.* **6**, 289–294 (1969).
67. Wetherill, G. W. *Geol. Soc. Am. Mem.* **97**, 513–519 (1966).
68. Lederer, C. M., Hollander, J. M. & Perlman, I. *Table of Isotopes* (Wiley, New York, 1970).
69. Tatsumoto, M., Knight, R. J. & Allegre, C. *Science* **180**, 1279–1283 (1973).
70. Goles, G. G., in *Handbook of Elemental Abundances in Meteorites* (ed. Mason, B.) 163–173 (Gordon and Breach, New York, 1971).
71. Morgan, J. W. in *Handbook of Elemental Abundances in Meteorites* (ed. Mason, B.) 517–528 (Gordon and Breach, New York, 1971).
72. Morgan, J. W. in *Handbook of Elemental Abundances in Meteorites* (ed. Mason, B.) 529–548 (Gordon and Breach, New York, 1971).

Proterozoic intraplate deformation in the light of South-east Asian neotectonics

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A comparison of lithospheric plate distortion in Proterozoic times with that known to be now taking place in SE Asia shows a close similarity between neotectonic and Precambrian crustal behaviour. Precambrian high grade metamorphic terrains have evolved by crustal thickening processes similar to those still active in China.

ONE of the outstanding geological problems concerns the stage in the Earth's history at which global tectonics could first be realistically described in terms of plate tectonics, and the ways in which plate behaviour has varied since that time.

It has been suggested^{1,2} that a fundamental change in crustal behaviour coincided with the beginning of the Proterozoic era (~2,600 Myr ago) which was characterised by large-scale linear structures, unknown in the early Archaean, and which reflected the development of tectonic régimes in the crust and mantle comparable in scale with those of the present.

It was subsequently pointed out³ that the large-scale transcurrent displacements which are an important element of Proterozoic tectonics, could not have taken place without the existence of a distinct lithosphere, with a lower bounding region accommodating translation relative to a substratum. A feature of the Proterozoic lithosphere³, apparently distinguishing it from that of the present day, was the large amount of deformation occurring within continental plates. While allowing that the rigidity of neotectonic plates may have been over emphasised, it was concluded³ that the Proterozoic lithosphere showed a real difference in mechanical behaviour, either because of differences in the forces acting on it or because of differences in its mechanical properties.

Recent work by Molnar and Tapponnier^{4–7} has, however,

demonstrated that neotectonic deformation of a large region (~5,000,000 km²) within the Eurasian plate, and comparable in several respects with the deformation of the Proterozoic continents, has accommodated intracontinental shortening of up to 2,000 km. The distortion in both the Proterozoic and neotectonic situations is expressed partly by large-scale conjugate transcurrent displacements, which lead to crustal widening as a response to the shortening, and partly by thrusting or other mechanisms which allow shortening to be accommodated by crustal thickening. Regional conjugate tectonics of this type are apparently uncommon, and require the existence of a regional stress system which is uniform, or which varies in a simple and systematic manner, on a regional scale. Highly ordered regional tectonics are unlikely in collisional orogenic belts because of the irregularity of the colliding continental margins⁸, and regionally uniform stresses and tectonics, on an areal as opposed to linear basis, are more likely to develop in the uniform interiors of continental plates, albeit in those parts of the interior adjacent to marginal orogens. Although it has been recognised that Proterozoic shear belts cannot be related to one another by the traditional orogenic concept³, the corollative conclusion about the special behaviour of the Proterozoic lithosphere needs to be re-examined in the context of the nonrigid behaviour of the Eurasian plate, and of conjugate tectonics. This re-examination is most conveniently carried out by comparing the displacements and stress systems in an extensive region of Proterozoic activity, the North Atlantic craton, with those now operating in China^{4,6}. The comparison is complicated by the difference in the tectonic level now exposed in the deeply eroded North Atlantic craton compared with the mainly surface expression of displacements to be seen in China. A prerequisite for the

comparison, therefore, is the recognition that seismic fault and thrust displacements, the basic observational features in China, represent the response of the uppermost 10–20% of the lithosphere to displacements along aseismic ductile shear zones which represent the primary displacements in a deforming lithosphere^{3,9–11}, and which are the basic observational units in Proterozoic rocks.

The tectonics of the North Atlantic craton, comprising the Precambrian of Central and Southern Greenland and the Lewisian of Scotland, are analysed below on three scales: scale I, area ~1,000,000 km²; scale II, area ~250,000 km²; scale III, area ~10,000 km². On scales II and III a well defined conjugate displacement system is evident, consistent with a regional maximum principal stress (σ_1) striking NW to NNW, and approximately horizontal. Although several elements on scale I are consistent with this pattern, a simple conjugate system is not apparent.

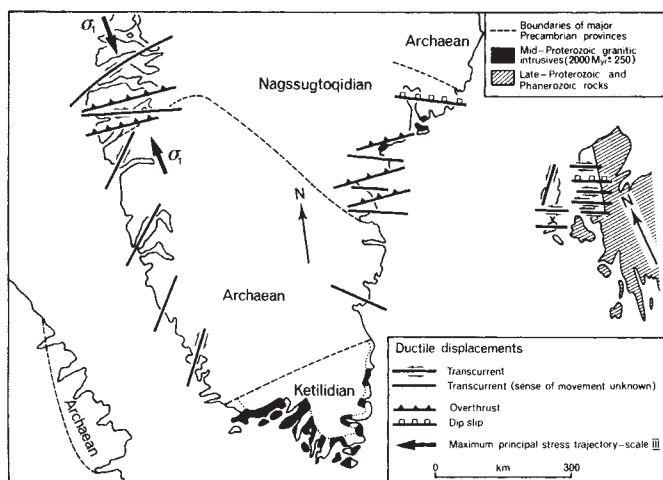


Fig. 1 Pre-Mesozoic reconstruction⁴⁵ of the North Atlantic region, with major Proterozoic ductile displacements in Greenland and Scotland⁴⁶ shown schematically. Subdivision of the Precambrian of central and south Greenland is shown together with areas of Mid-Proterozoic granitic rocks. Present geographic north shown separately in Greenland and Scotland.

Scale I

The Precambrian of this region (Fig. 1) with the exception of the Ketilidian belt in southern Greenland, consists of Archaean rocks modified by spaced ductile shear belts of Proterozoic age, and up to 40 km wide. In some parts the shear belts are closely spaced, and even the blocks between them show evidence of low Proterozoic strains. In other parts individual shear belts are sharply bounded by unmodified Archaean rocks. With rare exceptions the rocks are of amphibolite or granulite facies. The more obvious known zones of high Proterozoic strain are shown in Fig. 1, together with an indication of the sense of displacement where this is known. Figure 1 is incomplete insofar as some areas have either not been mapped, or are described in ways which would not enable shear zones to be identified. The figure is simplified because of the need, especially in eastern Greenland and Scotland, to represent numerous small displacements by single symbols. Displacements on the larger zones exceeds 100 km (ref. 3).

No simple conjugate system is apparent on this scale but some systematic features are evident: (1) transcurrent displacements strike either NE (030°–065°) or ESE (095°–130°); (2) in Greenland, where the sense of displacement is known the NE zones are usually sinistral and ESE zones dextral; (3) in Scotland the reverse of (2) is usual; (4) overthrust displacements are invariably directed towards SE or SSE.

Scale II

A regional system of conjugate fractures and displacements is most easily demonstrated by reference to the North Atlantic dyke swarm¹². The swarm occurs in Scotland, East and West Greenland, Labrador and possibly Norway (Fig. 2) and includes two dominant dyke sets which in W. Greenland strike NNE–SSW and ESE–WNW. The basaltic dykes are emplaced either within or parallel to minor (<200 m) ductile shear zones with mainly transcurrent displacements. There is evidence¹² that the two dyke sets were emplaced parallel to planes of high shear stress, rather than to the plane of minimum normal stress (σ_1 – σ_2 principal plane) usually associated with dyke emplacement, and that these planes represented a conjugate pair resulting from a horizontal NW–SE σ_1 , vertical σ_2 and NE–SW σ_3 axis. The direction of opening of both sets of dyke fissures is NE–SW, that is, parallel to σ_3 , rather than normal to dyke margins. These dykes provide convincing evidence for the existence of a uniform regional stress system. The dyke swarm post-dates the large-scale transcurrent displacements and pre-dates the overthrust displacements, except in East Greenland¹³ where overthrust displacements occurred both before and after emplacement of the dykes. Each set contains several hundreds of individual dykes, up to 100 m in width. Where the dyke concentrations are greatest (Fig. 2) dyke material comprises about 3% of the total rock¹⁵, representing a probable 20 km NE–SW crustal widening.

Scale III

The Holsteinsborg–Søndre Strømfjord area, lying between 66° and 67°N on the west coast of Greenland, has been investigated with the specific aim of determining Proterozoic displacements. The main tectonic element is the Ikertôq shear belt, a vertical zone, 40 km wide, of highly strained gneisses indicating dextral transcurrent displacement^{3,9,10,14} (Fig. 1). The strike of the shear zone is between 090° and 100°. Unmodified Archaean rocks adjacent to the shear zone, and in low deformation lacunae within it, are cut by smaller (up to 200 m) dextral shear zones parallel to the major zone, and by conjugate NE sinistral zones. The smaller shear zones are often preferred sites for dyke intrusion¹².

Post-dating the transcurrent movements are two major zones of ductile overthrusting^{1,14,15} (Fig. 1), 15 km and 10 km wide in horizontal outcrop, dipping 20° and 50° to the NNW. The horizontal shortening within one of these zones has been estimated as 65% along a NNW–SSE axis¹⁶. The emplacement of thrust nappes of supracrustal rocks post-dated the ductile

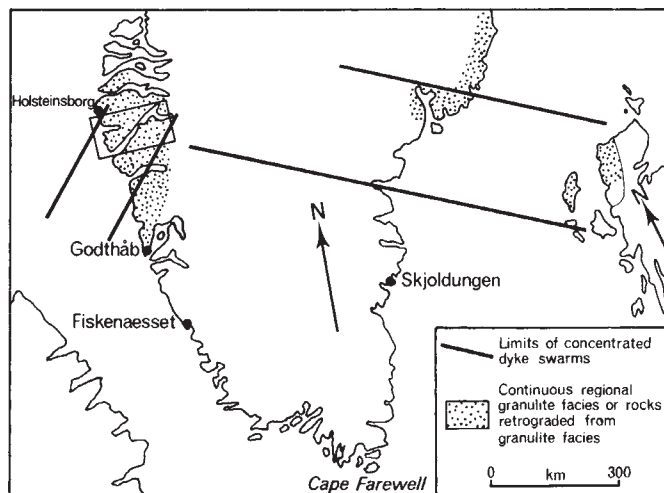


Fig. 2 Limits of the main concentrations of the two dyke sets comprising the North Atlantic swarm. Individual dykes parallel the limits shown. Areas of continuous granulite facies rocks, or rocks retrograded from granulite facies shown stippled. Holsteinsborg–Søndre Strømfjord area (scale III) indicated by box.

overthrusting but the movement direction was still towards S or SE¹⁷. Although the orientation of σ_1 during these overthrust movements was the same as that prevailing during the transcurrent shear displacements and dyke emplacement, an interchange of σ_2 and σ_3 was necessary to produce the overthrust displacements. The extended period over which dyke intrusion occurred¹⁸ was characterised in both Greenland¹⁹ and Scotland²⁰ by a close association between dyke intrusion and country rock deformation.

Other displacement phenomena in the Holsteinsborg district demonstrating a NW or NNW trending σ_1 include deformation fabrics in diorite dykes²¹, conjugate pseudotachylite generation surfaces, and brittle thrusts with pseudotachylite^{10,14}.

The stress trajectories were remarkably uniform in the Holsteinborg region during the formation of a wide range of displacement structures, over a period extending from about 2,600 to 2,000 Myr ago or later^{18,22}. A similar stress pattern over a wider area (scale II) is evident from both the distribution of the North Atlantic dyke swarm and individual elements of it, and features consistent with the same stress trajectories can be identified on scale I. Estimates of crustal shortening along a NNW-SSE axis on scale III suggest an approximately 50% shortening with approximately equal contributions from transcurrent and overthrust ductile shear displacements. No estimate of shortening on scales I and II is yet possible, although it is probably much less than 50%.

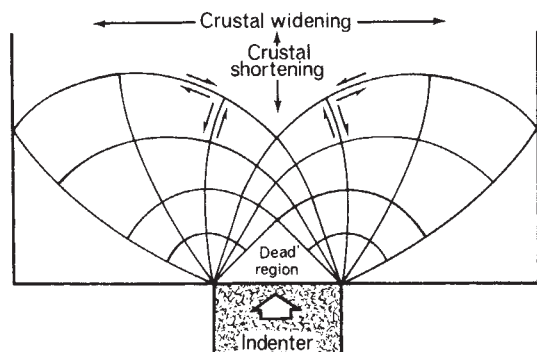


Fig. 3 Trajectories of maximum shear stresses (slip lines) for indentation model, with rigid indenter (shaded) quarter-width of rigid-plastic plate. Arrows show sense of movement on potential transcurrent displacements. Modified from Fig. 4b of ref. 6.

The regional stress trajectories derived from fault displacements in the Eurasian plate have been analysed⁶ in terms of a model incorporating a rigid-plastic plate (Eurasia) indented by a rigid die (India). Trajectories of maximum shear stresses for a version of this model in which the indenter is quarter-width of the indented plate, are shown in Fig. 3. Although the model is oversimplified it does provide a convincing theoretical framework for consideration of regional conjugate tectonics, despite a required difference in rheological properties of the colliding continental masses. A significant difference between the observed Precambrian structures and model relationships, is that the Proterozoic conjugate structures characteristically have a 120° conjugate angle in the σ_1 sector¹⁰ rather than the 90° conjugate angle of the model. Analytical problems apart, Molnar and Tappanier have convincingly demonstrated⁷ (1) widespread areal distortion within the Eurasian plate, (2) a regionally systematic pattern of transcurrent and thrust displacements producing significant crustal shortening, and (3) a close time relationship between continental collision and the onset of intraplate distortion. The first two of these have been shown to apply also to the North Atlantic region in the Proterozoic, and the possibility of the Proterozoic displacements also being collision effects should be considered.

Similarities between Proterozoic ductile overthrust tectonics and plate margin phenomena seen at higher levels in the Caledonian and Alpine belts were suggested¹, although later discounted³ because of the absence of a suture in central Greenland. The similarity between the essentially igneous Ketilidian

province in South Greenland and the Andean type of plate margin igneous assemblage has also been commented on¹. The Ketilidian igneous assemblage, of early Proterozoic age²⁴, includes a wide range of calc-alkaline rocks ranging from acid-volcanics to rapakivi granites, with associated appinitic basic intrusions. Some connection between Ketilidian activity and Proterozoic deformation further north has long been suspected²⁵ because of the similarity in age, but a direct causal relationship has not previously been suggested because the displacements occur up to 1,000 km from the Ketilidian boundary. However, the demonstration that displacements related to the Indian-Eurasian plate boundary occur up to at least 2,000 km from the Indus suture, shows that the Proterozoic displacements could be a consequence of a collision which also gave rise to the Ketilidian assemblage, the suture either being unrecognised in South Greenland or lying to the south of Cape Farewell. Not only is the Ketilidian boundary normal to the maximum principal stress trajectory derived from the displacement pattern, but a similar stress pattern, derived from ductile strains and conjugate dyke sets, has been described²⁶ from within the Ketilidian belt.

A more detailed application of the indentation model to the North Atlantic craton is possible if the distribution of Proterozoic structures is considered, in addition to their orientations. The Precambrian of southern and central Greenland has been regarded as consisting of a central Archaean block, bordered to the south by the Ketilidian belt and to the north by the Nagssugtoqidian belt (Fig. 1). The boundary of the Nagssugtoqidian is the southern limit of intense Proterozoic deformation and displacement, although most of the rocks within the belt are of Archaean origin^{27,28}. Proterozoic displacements within the Archaean block are more widely spaced and probably of smaller magnitudes than those within the Nagssugtoqidian belt: consequently the precise position of the Nagssugtoqidian boundary is to some extent arbitrary but notwithstanding the range of possible positions, a marked change in direction must occur under the Inland Ice (Fig. 1) if the boundary in East Greenland, dominated by SE striking structures, is to join that on the west coast where NE striking structures are dominant. The northern boundary of the Archaean block defined in this way has a similar disposition to that of the more dense parts of the two dyke sets which comprise the North Atlantic swarm (Fig. 2). The main area of intersection of the two dyke sets and the probable change in direction of the Nagssugtoqidian boundary lie at the apex of a triangular block of Archaean rocks, the western corner of which is represented by the restricted area of Archaean rocks in coastal Labrador²⁶. The Archaean triangle (Fig. 4) is thus bordered to the south by the Ketilidian belt and to the NW and NE by closely spaced shear belts and dyke swarms. The existence of such a relatively undeformed block between the collisional Ketilidian belt and the displacements and dyking also ascribed to the collision, is less paradoxical than it may at first appear, when seen in the context of an indentation model. Slip-line fields for indentation models vary with changes in boundary conditions defined by the relative sizes of indenting and indented blocks⁶, but a feature common to all models is a triangular region ahead of the indenter (Fig. 3) within which little displacement is likely to occur. If the Archaean block is related to such a region, several associated features are more easily understood, including the difference between the displacement directions in Greenland and Scotland. The region immediately beyond the apex of a 'dead' triangle is expected to be one of particularly complex deformation because of compatibility problems, and one where activation of both sets of conjugate slip planes is likely, as is found in the Holsteinsborg region. The decrease in normal stresses outside the limits of the 'dead' triangle⁶ would favour dyke emplacement, which requires the hydrostatic pressure in the dyke magma to exceed the minimum normal stress. More than any other feature of the North Atlantic craton, the disposition and orientation of the two sets of dykes, together with their observed association with

shear fractures, provide a requirement for, and impose rigid constraints on, a model of regional stress and displacement.

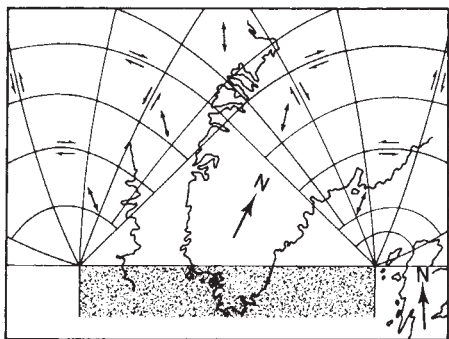


Fig. 4 Indentation model in relation to the North Atlantic craton with 'dead' triangle superimposed on the Archean, and indenter on the Ketilidian, showing potential transcurrent (split arrows) and over-thrust (double arrows) displacement directions.

A model of the type illustrated in Fig. 4 will inevitably be a gross over-simplification of the natural structures, but it should have a value in indicating the type of investigations which are needed in the Precambrian, and in showing the scale on which interpretations are to be made. The model overemphasises the significance of the crustal widening response to shortening relative to the crustal thickening response, the mechanism for which may be ductile overthrusting, ductile pure shear deformation, tectonic underplating of one continent by another, or some combination of these mechanisms. The profound effect of the thickening response in SE Asia is seen in the elevated topography of a large region, and an area of over 7,000,000 km² with a crustal thickness of more than 50 km (ref. 30). Crustal thickening on such a scale has profound effects on the subsequent thermal history of a region³¹, and the high heat flow observed in the uplifted deforming region of the Eurasian plate is a significant indication of this³². Uplift and complementary erosion are likely to continue in SE Asia until normal crustal thickness is re-established, when high grade metamorphic rocks, now at depths of 20–30 km, will be exposed at the surface. The high grade regions of the Precambrian shields, in contrast to the low-grade granite-greenstone belt terrains, have typically had 20–30 km of overburden removed by erosion^{33–35}. This great depth of erosion can only have been a consequence of areally widespread crustal thickening, as now seen in SE Asia. In the North Atlantic craton, the contrast between the lower pressure metamorphic assemblages of the Archean block and the higher pressure assemblages of the Nagssugtoqidian has previously been remarked upon, and accords with the lesser crustal thickening expected in the less deformed Archean triangle compared with that in the bordering regions. A similar conclusion can be drawn from the distribution of metamorphic facies in the North Atlantic craton (Fig. 2) where regions of uniformly granulite facies rocks, or those known to be retrograded from granulite facies, occur only outside or marginally within the Archean triangle, that is, regions subjected to greatest tectonic thickening. Granulite facies occurrences in the Fiskenaeset, Skjoldungen, and Cape Farewell areas (Fig. 2) are of a more local type. Further indications of the exposure of progressively deeper rocks, equilibrated at progressively higher temperatures, away from the Archean triangle, are provided by metamorphic assemblages in shear zones and by dyke relationships. Ductile shear zones usually retrogress granulite facies country rocks, except in the most northerly examples on both the west³⁶ and east³⁷ coasts of Greenland where granulite facies assemblages were stable during shear deformation at the level now exposed. Dykes of the North Atlantic swarm show clear evidence of intrusion into hot country rocks at the northern limits of their range on both east and west coasts in Greenland^{10,37}, and in Scotland²⁰, but towards the south show more

normal relationships with their cooler and presumably higher level country rocks.

Tectonic crustal thickening on the scale proposed is expected to lead to the large-scale production of granitic partial melt²⁰, but this seems not to have occurred, or at least has not been recognised. Apart from relatively small occurrences in eastern Greenland and in Scotland, Proterozoic displacements were not accompanied by granitic activity at the level now exposed, but the generation of large volumes of basic magma represented by the dyke swarms is of great thermal significance.

Intracontinental tectonic thickening of the crust is likely to produce permanent differences in crustal structure across the boundaries between thickened and nonthickened regions, similar to those shown to be characteristic of province boundaries in the Canadian shield²⁸. The Canadian examples are regarded as interplate boundaries, this interpretation being supported in the case of the Slave–Churchill boundary, which shows large-scale conjugate displacements³⁹, by application of an indentation model⁴⁰. Plate boundaries and intraplate tectonic boundaries, of the type represented by the Greenland Archean–Nagssugtoqidian boundary, are likely to have many common features, including gravity profiles and the presence of extensive areas of highly deformed gneisses.

Although intra-plate distortion occurs in only a small proportion of the present continental crust, in SE Asia and the western United States, a much higher proportion of the Proterozoic crust seems to have been affected because the high-grade regions form probably at least half of the shield areas. The neotectonic situation is perhaps unusual in that continental collision boundary represents only a small fraction of total plate boundary length. Another consideration must be the 2,000 Myr available for Proterozoic activity, although major displacements in the North Atlantic craton were probably completed, and erosion well advanced by 1,800 Myr (ref. 41). The concentrations of radiometric ages in the Precambrian into a few relatively short intervals⁴², and interpreted in terms of Chelogenic cycles⁴³, may represent a small number of widespread collisional events, with activity in the North Atlantic craton representing a small part of one such event.

It therefore remains uncertain whether the Proterozoic lithosphere behaved in a way significantly different from that of the Phanerozoic, but at this stage the similarities of behaviour are much more striking than the possible differences and to such a degree that some Proterozoic phenomena may be used as a basis for interpreting modern plate behaviour, rather than the reverse. One notable difference is that the late Archean–early Proterozoic lithosphere was mechanically more isotropic than that of later times, and its mechanical behaviour likely to conform more closely to simple deformation models. Large-scale ductile shear zones, once formed, may persist as permanent ductile weaknesses in the lithosphere⁹, owing to the reduced grain size, allowing renewed displacement⁴⁴ along directions significantly different from those predicted by models assuming a mechanically isotropic continental lithosphere. As the continental lithosphere has become increasingly anisotropic through time, the analysis of more recent intracontinental tectonics is correspondingly more difficult, in contrast to the mechanical simplicity of both the early Proterozoic continents and present day oceans.

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1. Bridgwater, D., Escher, A. & Watterson, J. *Phil. Trans. R. Soc. A* **273**, 513–533 (1973).
2. Sutton, J. & Watson, J. V. *Nature* **247**, 433–435 (1974).
3. Bak, J. et al. *Nature* **254**, 566–569 (1975).
4. Tappinier, P. & Molnar, P. *J. geophys. Res.* **82**, 2905–2930 (1977).
5. Molnar, P. & Tappinier, P. *Science* **189**, 419–426 (1975).
6. Molnar, P. & Tappinier, P. *Geology* **5**, 212–216 (1977).
7. Molnar, P. & Tappinier, P. *Scient. Am.* **236**, 30–41 (1977).

8. Dewey, J. F. & Burke, K. *Geology* **2**, 57–60 (1974).
9. Watterson, J. *Nature* **253**, 520–522 (1975).
10. Watterson, J. *Rapp. Grønlands geol. Unders.* **65**, 33–37 (1974).
11. Grocott, J. *J. geol. Soc. Lond.* **133**, 257–263 (1977).
12. Escher, A., Jack, S., & Watterson, J. *Phil. Trans. R. Soc. A* **280**, 529–539 (1976).
13. Andrews, J. R., Bridgwater, D., Gormsen, K., Gulson, B., & Watterson, J. in *The Lewisian of Scotland and Related Rocks of Greenland* (eds. Park, G. & Tarney, J.) (Keele University Press, 1973).
14. Grocott, J. thesis, Liverpool Univ. (1977).
15. Escher, A., Escher, J. & Watterson, J. *Can. J. Earth Sci.* **12**(2), 158–173 (1975).
16. Escher, A. & Watterson, J. *Tectonophysics* **22**, 223–231 (1974).
17. Diggins, J. & Talbot, C. *Rapp. Grønlands geol. Unders.* **65**, 37–39 (1974).
18. Kalsbeek, F., Bridgwater, D. & Zeck, H. *Can. J. Earth Sci.* (in the press).
19. Bridgwater, D., Escher, A. & Watterson, J. in *The Lewisian of Scotland and Related Rocks of Greenland* (eds. Park, G. & Tarney, J.) (Keele University Press, 1973).
20. Park, R. G. & Gresswell, D. in *The Lewisian of Scotland and Related Rocks of Greenland* (eds. Park, G. & Tarney, J.) (Keele University Press, 1973).
21. Davidson, L. & Park, G. *J. Geol. Soc. Lond.* (in the press).
22. Chessex, R., Delaloye, M., Gulacar, F. & Escher, A. *Fortschr. Miner.* **50**, 60–61 (1973).
23. Allaart, J. H. in *The Geology of Greenland* (eds. Escher, A. & Watt, S.) (Geological Survey of Greenland, 1976).
24. Petersen, S., Larsen, O., Bridgwater, D. & Watterson, J. *Rapp. Grønlands Geol. Unders.* **66**, 12–20 (1974).
25. Berthelsen, A. in *Geology of the Arctic* (ed. Raasch, G. O.) (University of Toronto Press, 1961).
26. Watterson, J. *Meddr. Grønland* **185**, 1–104 (1968).
27. Escher, A., Sørensen, K., & Zeck, H. in *The Geology of Greenland* (eds. Escher, A. & Watt, S.) (Geological Survey of Greenland, 1976).
28. Bridgwater, D. in *The Geology of Greenland* (eds. Escher, A. & Watt, S.) (Geological Survey of Greenland, 1976).
29. Bridgwater, D., Escher, A., Jackson, G. D., Taylor, F. C. & Windley, B. F. *Am. Ass. Pet. Geol. Mem.* **19**, 96–116 (1973).
30. Cummings, D. & Shiller, G. I. *Earth-Sci. Rev.* **7**, 97–119 (1971).
31. England, P. C. & Richardson, S. W. *J. Geol. Soc. Lond.* **134**, 201–213 (1977).
32. Clark, S. P. & Jäger, E. *Am. J. Sci.* **267**, 1143–1160 (1969).
33. Davidson, L. thesis, Liverpool Univ. (1978).
34. Wells, P. R. A. *Contr. Mineral. Petrol.* **56**, 229–242 (1976).
35. O'Hara, M. J. *J. geol. Soc. Lond.* **134**, 185–200 (1977).
36. Bak, J., Korstgård, J. & Sørensen, K. *Tectonophysics* **27**, 191–209 (1975).
37. Bridgwater, D. *et al. Rapp. Grønland geol. Unders.* **85**, 74–83 (1977).
38. Gibb, R. A. & Thomas, M. D. *Nature* **262**, 199–200 (1976).
39. Gibb, R. A. & Thomas, M. D. *Tectonophysics* **38**, 211–222 (1977).
40. Gibb, R. A. *Nature* **271**, 50–52 (1978).
41. Morgan, G. *Nature* **259**, 382–385 (1976).
42. Gastil, G. *21st Session Int. Geol. Cong.* **9**, 162–169 (1960).
43. Sutton, J. *Nature* **198**, 731–735 (1963).
44. Watson, J. *Phil. Trans. R. Soc. A* **280**, 629–640 (1976).
45. Bullard, E. C., Everett, J. E., & Smith, A. C. *Phil. Trans. R. Soc. A* **258**, 41–51 (1965).
46. Davies, F. B. *J. geol. Soc. Lond.* **132**, 543–544 (1976).

Isolation and partial sequence of recombinant plasmids containing human α -, β - and γ -globin cDNA fragments

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Human globin cDNA-derived recombinants with plasmid pCR1 have been prepared for use as specific hybridisation probes and for the partial sequencing of α -, β - and γ -globin genes.

TO analyse the molecular biology of developmental and pathological systems in humans, one must investigate not only the expression of single genes, but also that of families of genes. One of the most interesting and most easily studied human gene families specifies the β -like globin chains. The gene arrangement is known, and the four β -like genes are thought to be closely linked¹. There is a switch during foetal and neonatal development from γ -globin synthesis to β -globin synthesis, while the synthesis of δ -globin remains at a low level throughout². There is evidence of different half lives for the otherwise similar δ and β -globin mRNA (ref. 3). Finally, there is a wide spectrum of genetic disease, the thalassaemias, in which the expression of the globin genes is affected, and which have been shown to be due to lesions at any one of several levels: gene deletion, lack of transcription, faulty post-transcriptional processing or translation defects^{4–8}. A similar α -gene family exists, although developmental switches in this case are less well understood⁹. There is also regulated expression of the amounts of α -globin and β -globin, a useful model for coordinate gene expression at the molecular level¹⁰.

Preparation and characterisation of chimaeric plasmids

To study the problems listed above, it is essential to prepare nucleic acid probes which are specific for each globin gene. So far, this has been done using either mRNA or cDNA. Preparation of total globin mRNA, and thus its complementary copy, is no longer difficult, but purifying a sequence complementary to only one of the globin genes poses problems, particularly if more than a very small amount of probe is required^{11,12}. To meet the needs for complete purity and large

amounts of these probes, we have prepared chimaeric plasmids in which a double-stranded DNA copy of human globin foetal mRNA has formed a recombinant with plasmid pCR1 (ref. 13).

The plasmids that have been isolated have been characterised by 'Grunstein-Hogness' hybridisation on a filter to purified polynucleotide kinase-labelled globin mRNA (refs 14, 15), then by solution hybridisation in plasmid excess to chain-specific cDNAs. The plasmids have also been characterised by restriction mapping, and in two cases the globin cDNA length confirmed by excision and direct measurement.

Plasmids were selected containing α -, β - and γ -globin gene sequences and in each case the plasmid selected contained a cDNA-derived sequence greater than half the length of the mRNA. The availability of this family of human globin gene sequences should facilitate detailed analysis of normal globin gene arrangement and the causes of dysfunction, particularly in thalassaemia.

Human globin messenger RNA was prepared using two cycles of oligo dT cellulose affinity chromatography (Collaborative Research, grade T3) using 'normal' adult reticulocytes (mRNA (α , β)), neonatal exchange transfusion blood (mRNA (α , β , γ)), and blood from a previously studied double heterozygote $\beta_0/\delta\beta_0$ thalassaemic with no mRNA (β) in circulating red blood cells (mRNA (α , γ)) (ref. 16). Messenger RNA for clone identification was used without further purification.

A sample of mRNA (α , γ , β) was used to prepare double-stranded cDNA for insertion into plasmids^{17–21}. This mRNA was first prepared by affinity chromatography, as above, treated with ribonuclease-free DNase (Worthington) and then centrifuged on a sucrose gradient; only material sedimenting at 9S was collected. Complementary DNA was prepared using RNA-dependent DNA polymerase. The cDNA was treated with alkali to remove RNA, neutralised and used as a template for the synthesis of a second, complementary strand with RNA-dependent DNA polymerase. The double-strand product was treated with S1 nuclease, elongated with terminal transferase, and a poly dA sequence was added to the 3' termini of the double-stranded cDNA using the method of Maniatis^{19,20}.

Plasmid identification and hybridisation to purified globin cDNA

Plasmid pCR1 was purified and cleaved at the single *Eco*RI restriction site, and the 3' termini were extended with poly dT sequences using terminal transferase²⁰. 100 ng of tailed plasmid were mixed with 2 ng of tailed double-stranded cDNA, annealed, and used to transfect *Escherichia coli* HB101. The molar ratio of plasmid to insert was 2.3:1. The number of recombinant clones obtained in three experiments, as judged by kanamycin resistance, was 37, 70 and 175. These were picked and plated out in arrays and sufficient cells grown to allow transfer to several replica Millipore HAWP filters. All the transfections and growth of recombinant clones before positive identification were carried out under UK category 3 biohazard containment conditions in the Department of Biochemistry, Imperial College, as advised by the UK Genetic Manipulation Advisory Group²².

Replica Millipore filters were prepared for hybridisation to appropriate (³²P)-labelled mRNAs essentially by the method of Grunstein and Hogness¹⁴. The mRNAs were cleaved into fragments approximately 100 nucleotides long by brief exposure to alkali before terminal labelling with polynucleotide kinase¹⁵. Each filter was hybridised to 2.5×10^6 d.p.m. of (³²P)-mRNA in a volume of 1.5 ml in 50% formamide hybridisation buffer for 18 h at 42°C.

After autoradiography of the filters, a set of clones hybridising strongly with (³²P)-mRNA (α , γ , β) and not scoring with (³²P)-mRNA (α , β) was identified as being most likely to contain γ -globin cDNA inserts. Another set scoring with all

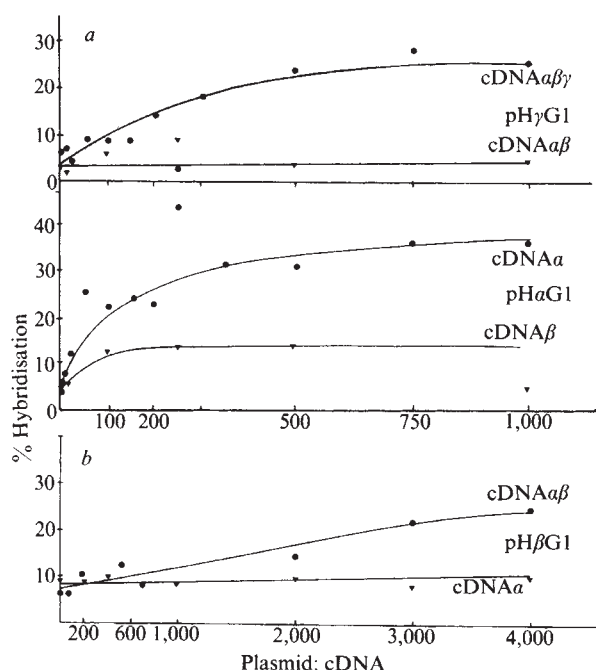


Fig. 1 cDNA-hybridisation curve of globin chimaeric plasmids pHαG1, pHβG1 and pHyG1. *a*, Hybridisation of pHyG1 to cDNAαβγ and cDNAαβ, and of pHαG1 to cDNAα and cDNAβ. *b*, Hybridisation of pHβG1 to cDNAαβ and cDNAα. The plasmid DNA was sheared to approximately 500–700 base pairs and was hybridised at increasing concentrations to 0.2 ng of the indicated cDNA probes in 2 μl formamide hybridisation buffer (50% formamide, 0.5 M NaCl, 0.25 M HEPES pH 7.5, 0.01 M EDTA), sealed into capillaries, boiled for 5 min and then left for 60 h at 42°C. The % of hybridisation was assayed by S1 nuclease treatment as described by Harrison *et al.*²⁹. The cDNA probes were prepared using reverse transcriptase as described by Jackson *et al.*³⁰. The cDNAα and cDNAβ probes were prepared by hybridisation of cDNAαβ to β₀mRNA in excess and separation of the single and double stranded nucleic acid by hydroxyapatite chromatography⁵.

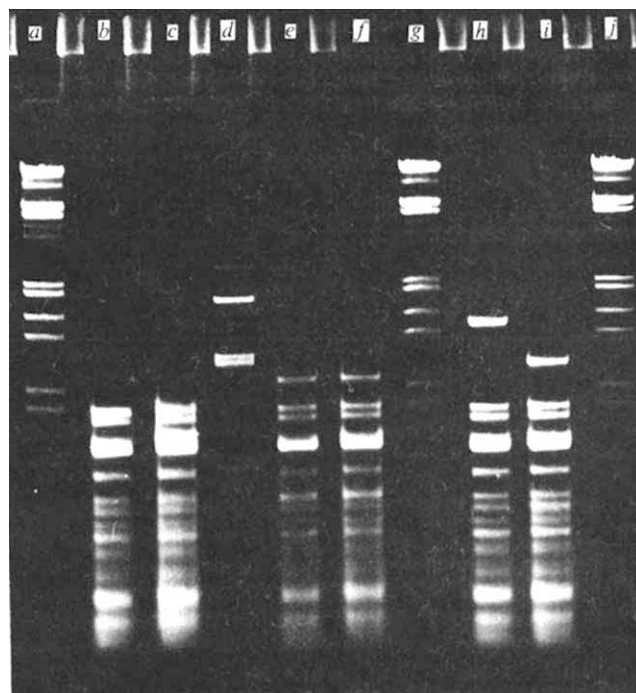


Fig. 2 Digestion of pCR1, pHαG1, pHyG1 with *Hpa*II and *Eco*RI plus *Hpa*II. Track *a*, 1.0 μg λC1857 digested with *Eco*RI and *Hind*III. Track *b*, 1.0 μg pCR1 digested with *Hpa*II. Track *c*, 1.0 μg pCR1 digested with *Hpa*II plus *Eco*RI. Track *d*, 0.3 μg SV40 digested with *Hind*III. Track *e*, 1.0 μg pHαG1 digested with *Hpa*II. Track *f*, 1.0 μg pHαG1 digested with *Hpa*II plus *Eco*RI. Track *g*, as *a*. Track *h*, 1.0 μg pHyG1 digested with *Hpa*II. Track *i*, 1.0 μg pHyG1 digested with *Hpa*II plus *Eco*RI. Track *j* as *a*. *Hpa*II digests were carried out on 2 μg plasmid DNA in 20 μl of 6 mM Tris, 6 mM 2-mercaptoethanol, pH 7.5, containing 0.5 units of enzyme. Digestion was at 37°C for 3.5 h. The sample was then split and half kept in ice. To the other half, 1 μl of 100 mM Tris, 500 mM NaCl, 100 mM MgCl₂, pH 7.5, and two units of *Eco*RI were added and digested at 37°C for a half hour. The reactions were then stopped with 1 μl of 0.5 M EDTA. The whole sample was run in a 2.0% Agarose (Miles) gel. Running buffer was 40 mM Tris, 20 mM NaOAc, 2 mM EDTA, pH 7.5, and electrophoresis allowed to proceed for 4 h at a voltage gradient of 6 V cm⁻¹. The gel was then stained with ethidium bromide and the ultraviolet induced fluorescence photographed on Ilford FP4 using a Wratten 25A red filter.

three probes mRNA (α , γ , β), mRNA (α , β), and mRNA (α , γ) was identified as most likely to contain α -globin inserts. Small cultures of colonies hybridising most strongly on filters were grown and DNA was prepared from the supernatant after pelleting the cell debris and the bulk of the chromosomal DNA. This was sonicated to a size of approximately 400 nucleotide pairs and then hybridised to purified globin cDNA probes at a DNA:cDNA ratio of 100,000:1. The probes used were cDNA (α , β) and cDNA (α , γ , β), prepared from purified globin mRNA from adult and neonatal reticulocyte rich blood, respectively, and cDNA (α) and cDNA (β) prepared using cross-hybridisation with thalassaemic mRNA as previously described²³. In every case the plateau hybridisation value obtained is a compound of the purity of the probe with respect to the globin sequence studied and the length of the insert.

Of the total 282 clones picked as kanamycin-resistant, 88 scored sufficiently strongly with (³²P)-mRNA to be characterised further; of these, 58 have been tentatively identified as containing γ -globin cDNA inserts, 15 as α -globin cDNA inserts, and 15 as β -globin cDNA inserts. The other clones did not hybridise to (³²P) ribosomal RNA and are thought to contain short globin cDNA inserts. The starting mRNA contained globin mRNA sequences in the approximate ratio α : β : γ = 55:12:32 (ref. 24). The ratio of clones obtained is

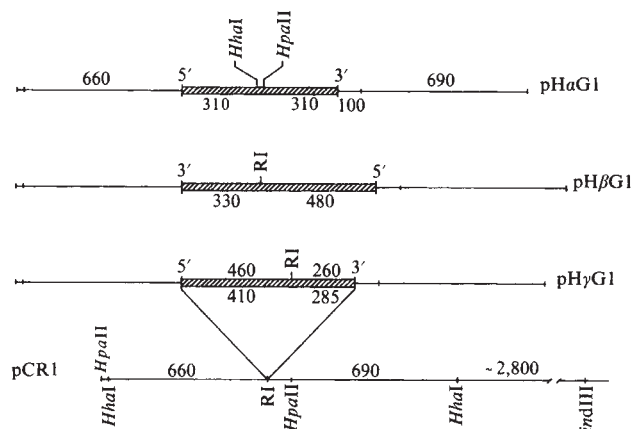


Fig. 3 Restriction map around the site of insertion of plasmids pCR1, pHαG1, pHβG1 and pHγG1. [RI] indicates the two halves of the *EcoRI* site of insertion. Approximate map distances are given in base pairs. The *HpaII* and *HhaI* sites in pHαG1 can be calculated to be about 30 base pairs apart, but their order relative to other restriction sites cannot be established from the restriction data presented in Fig. 2. The orientation of the inserted DNA in pHαG1 is deduced from the nucleotide sequence shown in Fig. 5. The two sets of map distances presented for pHγG1 are derived from experiments excising the inserted DNA from pCR1 with *HpaII* (above) and *HhaI* (below). The inserted DNA sequences in each plasmid (including the poly dAdT tracks) are signified by shading.

consistent with the previous results from other groups^{20,25} that more β-globin cDNA than α-globin cDNA clones are obtained for a given mRNA with approximately equal amounts of the two globin types. Human γ-globin cDNA behaves like β-globin cDNA in its preferential incorporation into plasmids.

Plasmids pHαG1, pHβG1 and pHγG1 were chosen as containing cDNA inserts of α-, β- and γ-globin gene sequences, respectively, and were grown in 1-litre culture under UK category 2 biohazard containment conditions, as they had been identified by two independent techniques as containing a known protein-coding sequence for a nontoxic protein. Approximately 2 mg plasmid DNA was obtained from a 1-litre culture after plasmid amplification with chloramphenicol (Sigma). It was found essential to use chloramphenicol rather than the clinically used chloramphenicol succinate to obtain successful amplification. The plasmid DNA from the gradients was examined with the electron microscope, and was found to be entirely in the form of supercoiled or open circles.

The plasmid was now available in sufficient amount to hybridise to purified globin cDNAs at various plasmid DNA:cDNA ratios; the hybridisation curves obtained for plasmids pHαG1, pHβG1, and pHγG1 are shown in Fig. 1, and demonstrate that the assignments are correct.

Partial sequencing of recombinant plasmids

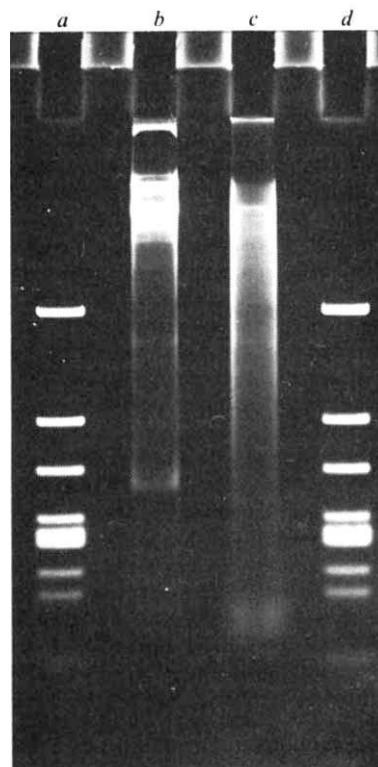
Each plasmid was then treated with a number of restriction enzymes: *HhaI*, *HpaII* and *EcoRI* proved particularly useful. It was found that *HhaI* and *HpaII* digest plasmid pCR1 to give a large fragment containing the region where insertion occurs (the original *EcoRI* site). *HhaI* (sequence cleaved, GCGC), *HpaII* (sequence cleaved, CCGG) and *HindIII* (sequence cleaved, AAGCTT) (ref. 26) cleave the α-globin cDNA insert, and do not cleave the β- or γ-globin cDNA inserts. *EcoRI* cleaves both the β- and γ-globin cDNAs at a single site, and does not cleave the α-globin cDNA insert.

It is possible to derive restriction site maps of the inserted

fragments by comparison of fragment patterns generated from chimaeric and non-chimaeric plasmids. Figure 2 shows this approach using *HpaII*. Track *b* is the fragment pattern for pCR1 with this enzyme. A single fragment at 790 base pairs is removed by digestion with *EcoRI* (track *c*) to generate a new band at 690 base pairs. This indicates that the single *EcoRI* site is 100 base pairs from one end of a 790-base pair *HpaII* fragment. The α plasmid gives a pattern (track *e*) with two new bands at 1,000 base pairs and 440 base pairs (this band runs at the same mobility as a pCR1 fragment, but its presence is proved by microdensitometry). This pattern may be interpreted by assuming that there is an *HpaII* site within the inserted DNA giving rise to the two new fragments. Thus, the 1,000-base pair fragment contains 690 base pairs of pCR1 and 310 base pairs of inserted DNA, the 440 fragment contains 100 base pairs of plasmid and 340 base pairs of inserted DNA. This enables the map in Fig. 3 to be derived. The γ plasmid gives a pattern with one new 1,500-base pair fragment (Fig. 2, track *h*). This consists of 790 base pairs of pCR1 plus 710 base pairs of inserted DNA. There is no internal *HpaII* site. Subsequent digestion with *EcoRI* breaks this new fragment to give two new bands (Fig. 2, track *i*) and the location of the *EcoRI* site is established in a fashion directly analogous to the α *HpaII* site. The β plasmid gave a pattern of fragments that was very similar to the pHγG1: a new band at 1,550 base pairs was visible, indicating an inserted DNA fragment of 760 base pairs. On digestion with *EcoRI*, this fragment was split to give new bands at 1,000 base pairs and 550 base pairs (data not presented).

Similar experiments were also carried out using *HhaI* to give the final maps shown in Fig. 3. There is ambiguity in the α *HhaI* map where the possible location of a second *HhaI* site remains obscure. The single *EcoRI* site in pHβG1 and pHγG1 allows

Fig. 4 Excision of the cDNA sequences from pHγG1 with S1 nuclease. Tracks *a* and *d*, *HaeIII* digested SV40 DNA. Track *b*, 1.0 μg pHγG1 DNA digested with 24 units S1 nuclease (Sigma) in 20 μl of 45% formamide, 30 mM NaOAc, 1 mM ZnSO₄, 0.2 M NaCl, pH 4.5, for 30 min at 55°C. Track *c*, 1.0 μg of pCR1 digested as *b*. The whole reaction mix is run on a 2.0% Agarose gel as described in the legend to Fig. 5. The prominent band seen in *b* is the inserted cDNA.



a tentative statement on the orientation of the inserted DNA. The cDNA β sequence is known²⁷ and has a single *EcoRI* site toward the 3'-end at the amino acid 121–122. Thus, the asymmetric distribution of DNA to the left and right suggests that the cDNA is inserted as shown in Fig. 3. Similar arguments apply to pH γ G1. Inspection of the protein sequence indicates only one possible *EcoRI* site in the coding sequence, again at 121–122. What has not been established from the restriction map alone is the absolute orientation of the *HpaII/HhaI* fragments within the whole plasmid.

The size of DNA which has been inserted into a plasmid using poly dA-poly dT tailing, as in this case, can also be estimated by excising the insert using S1 nuclease in conditions where only the poly dA-poly dT regions melt²⁸. This was done for the γ -globin plasmid pH γ G1 and the results are shown in Fig. 4. The size of the inserted sequence is 500 nucleotide pairs. As this does not include the dA and dT sequences, it is in excellent agreement with the restriction data. Similar experiments for pH β G1 indicate an inserted sequence of 540 base pairs (data not given).

All three plasmids were further characterised by direct sequence analysis around a restriction site in the coding region of the inserted DNA. The results (Fig. 5) confirm the assignments made from the hybridisation data and further differentiate between pH β G1 and pH γ G1. Sequencing was carried out

75	80	85	90	
AspMetProAsnAlaLeuSerAlaLeuSerAspLeuHisAlaHisLysLeu				α -globin
GACAUGCCCAACGCGCUGUCGCCCGAGGACGACGCGACAAGCUU				pH α G1
				<i>HindIII</i>
95	100			
ArgValAspProValAsnPheLysLeuLeu				α -globin
CGGGUGGACCCGCGUCAACUUAAGCUCCUA				pH α G1
105	110	115	120	
LeuLeuGlyAsnValLeuValCysValLeuAlaHisHisPheGlyLysGlu				β -globin
CUCCUGGGCAACGUGUGGUCUGUGUGGCCCCAUCACUUGGCAAA-GAA				pH β G1
				R1
105	110	115	120	
LeuLeuGlyAsnValLeuValThrValLeuAlaIleHisPheGlyLysGlu				γ -globin
CUCCUGGGAAAUUGUGUGUGUACCGUUUUGGCAAUCCAUUCGGGCAAA-GAA				pH γ G1
				R1
125	130	135		
PheThrProGluValGlnAlaSerTrpGlnLysMetValThr				γ -globin
UUCACCCUGAGGUGCAGGCUUACUGGCAGAGAUGGUGACU				pH γ G1
				<i>MboII</i>

Fig. 5 Partial nucleotide sequence of plasmids pH α G1, pH β G1 and pH γ G1. Plasmids pH β G1 and pH γ G1 were restricted with *EcoRI* and labelled at their 5' termini with γ -³²P-ATP using polynucleotide kinase (PL-Biochem). Secondary restriction was with *HindIII* which cleaves pCR1 at a single site and does not cleave the inserted DNA. The two resulting restriction fragments were separated on preparative 0.6% agarose gels. After isolation from this gel, each labelled fragment was divided into aliquots and subjected to the four-base-specific cleavage reaction described by Maxam and Gilbert³². The products were fractionated on a 20% acrylamide, 7 M urea, gel. Autoradiography was carried out at -70°C using Fuji RX film and Ilford fast tungstate intensifying screens. pH α G1 was cleaved with *HindIII* before labelling with polynucleotide kinase. Secondary restriction was carried out with *KpnI*, yielding four labelled fragments, two of which consist solely of pCR1 sequences. The fragments were separated on a 0.6% agarose gel and only the smallest and largest fragments corresponding to approximately 1.45 and 7.50 kilobases, respectively, were isolated. After isolation, the base-specific reactions and fractionation of cleavage products were carried out as for pH β G1 and pH γ G1 plasmids. Sequences read from the autoradiographs are presented in the RNA form of the coding strand of the inserted DNA. Nucleotides shown underlined signify an ambiguity at this position. Nucleotides lying within the restriction sites of *EcoRI*, *HindIII* are deduced from the known specificity of these restriction endonucleases²⁶.

according to the method of Maxam and Gilbert³² around the *HindIII* site in the pH α G1 inserted sequence and around the *EcoRI* site in the pH β G1 and pH γ G1 inserted sequences.

Sequencing of the α plasmid shows that out of the five possible *HindIII* sites predicted for the human α globin protein sequence, the cDNA is cleaved at a site spanning the amino acids 90–91. The nucleotide sequences derived from pH β G1 and pH γ G1 confirm that the *EcoRI* site lies at amino acids 120–121, and in addition, the nucleotide sequence for the β globin agrees fully with sequences previously published^{27,34}. The sequencing studies also confirm the proposed orientation of each inserted sequence relative to known pCR1 restriction sites.

The construction of human globin cDNA plasmids will be of use in the study of human globin gene cluster. Thus, the plasmids may be used as specific hybridisation probes for purification of genomic globin DNA sequences by affinity chromatography, for restriction mapping after the method of Southern (ref. 34 and Flavell *et al.*, in preparation), or for screening recombinant DNA molecules containing genomic globin DNA sequences.

After this work had been completed, a paper was published³⁵ describing the construction of similar human globin cDNA-derived recombinants with plasmid pMB9, and reporting the sequence of the β -globin gene insert. Our data are compatible with, and extend those, reported by Wilson *et al.*³⁵.

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- Weatherall, D. J. & Clegg, J. B. *The Thalassemia Syndromes*, 2nd edn (Blackwell, Oxford, 1972).
- Wood, W. G. *Br. med. Bull.* **32**, 282–287 (1976).
- Clegg, J. B. & Weatherall, D. J. *Lancet* **ii**, 133–135 (1974).
- Ottolenghi, S. *et al. Cell* **9**, 71–80 (1976).
- Tolstoshev, P. *et al. Nature* **259**, 95–98 (1976).
- Comi, P. *et al. Eur. J. Biochem.* **79**, 617–622 (1977).
- Kan, Y. W., Holland, J. P., Dozy, A. M. & Varmus, H. E. *Proc. natn. Acad. Sci. U.S.A.* **72**, 5140–5144 (1975).
- Conconi, F. *et al. Nature* **254**, 256–259 (1975).
- Weatherall, D. J. & Clegg, J. B. *A. Rev. Genet.* **10**, 157–178 (1976).
- Lodish, H. F. & Jacobson, M. J. *biol. Chem.* **247**, 3622–3629 (1972).
- Ottolenghi, S. *et al. Nature* **251**, 389–392 (1974).
- Kan, Y. W. *et al. Nature* **255**, 255–256 (1975).
- Armstrong, K. A., Hershfield, V. & Helinski, D. R. *Science* **196**, 172–174 (1977).
- Grunstein, M. & Hogness, D. S. *Proc. natn. Acad. Sci. U.S.A.* **72**, 3961–3965 (1975).
- Matzels, N. *Cell* **9**, 431–438 (1976).
- Ottolenghi, S. *et al. Proc. natn. Acad. Sci. U.S.A.* **72**, 2294–2299 (1975).
- Rabbitts, T. H. *Nature* **260**, 221–224 (1976).
- Rougeon, F., Kourilsky, P. & Mach, B. *Nucleic Acid Res.* **2**, 2365–2378 (1975).
- Elfratiadis, A., Kafatos, F. C., Maxam, A. M. & Maniatis, T. *Cell* **7**, 279–288 (1976).
- Maniatis, T., Kee, S. G., Elfratiadis, A. & Kafatos, F. C. *Cell* **8**, 163–182 (1976).
- Wilson, J. T., Forget, B. G., Wilson, C. B. & Weissman, S. M. *Science* **196**, 200–202 (1977).
- Williams, R. E. O. *Report of the Working Party on the Practice of Genetic Manipulation* (HMSO, London, 1976).
- Williamson, R. *Br. med. Bull.* **32**, 246–250 (1976).
- Old, J. *et al. Cell* **8**, 13–28 (1976).
- Rougeon, F. & Mach, B. *J. biol. Chem.* **252**, 2209–2217 (1976).
- Roberts, R. J. *CRC Critical Rev. Biochem.*, 123–164 (1976).
- Marotta, C. A., Wilson, J. T., Forget, B. G. & Weissman, S. M. *J. biol. Chem.* **252**, 5040–5053 (1977).
- Hofstetter, H., Schambeck, A., Van Den Berg, J. & Weissmann, C. *Biochim. biophys. Acta* **454**, 587–591 (1976).
- Harrison, P. R., Birnie, G. D., Hell, A., Humphries, S., Young, B. D. & Paul, J. *J. molec. Biol.* **84**, 539–554 (1974).
- Jackson, J. F., Tolstoshev, P., Williamson, R. & Hendrick, D. *Nucleic Acid Res.* **3**, 2019–2026 (1976).
- Laskey, R. A. & Mills, A. D. *FEBS Lett.* **82**, 314–316 (1977).
- Maxam, A. M. & Gilbert, W. *Proc. natn. Acad. Sci. U.S.A.* **74**, 560–564 (1977).
- Schroeder, W. A. *et al. Proc. natn. Acad. Sci. U.S.A.* **60**, 537–544 (1968).
- Southern, E. M. *J. molec. Biol.* **98**, 503–517 (1975).
- Wilson, J. T. *et al. Nucleic Acid Res.* **5**, 563–581 (1978).

letters to nature

Interferometric observations of weak radio flares from a red dwarf star

MEASUREMENT of the radio emission associated with flares on M-type red dwarf stars has been difficult because of the sporadic and transient nature of the phenomena, and the danger of confusion with terrestrial sources of emission. The existence of detectable radio emission from the flares was first established by integration and correlation with the optical flares^{1,2} but in several thousand hours of observations there are only a few cases where the existence of individual flares of long duration has been unambiguous³⁻⁵. We have used a long baseline interferometer for such measurements, and report here observations which yielded clear records of weak radio flares from the star YZ CMi.

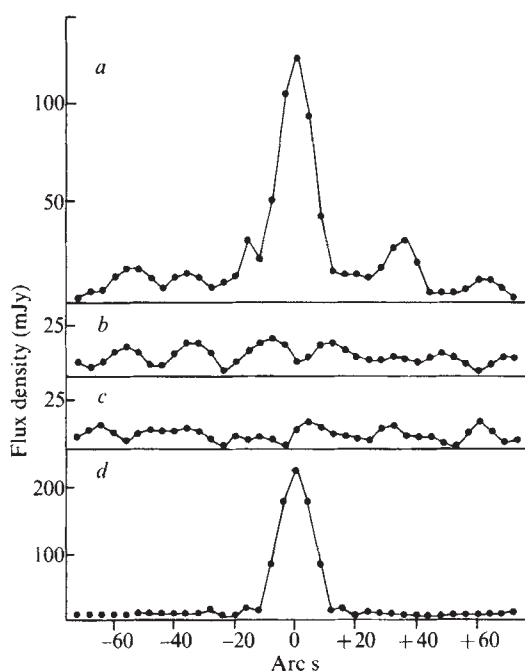


Fig. 1 Fourier transforms of the 30 s data points taken in 1 h. *a*, On the star channel during the maximum of the flare on 18 December 1977. *b*, On a blank sky channel at the same time. *c*, On the star channel at a different time. *d*, On a channel directed at the reference source.

These observations were made at a frequency of 408 MHz (bandwidth 4 MHz) with the MK 1A 76 m radio telescope at Jodrell Bank and the 25 m telescope at Defford. Both telescopes were equipped with r.h. circularly polarised feeds. The baseline of 127 km gives a lobe-width at this frequency of 1.2 arc s. The primary integration time was 30 s but, using a background source as a phase reference⁶, integrations of indefinite length could be carried out. An individual output channel of the interferometer is sensitive to an area of sky approximately 8×1.5 arc min² after a 30-s integration. As this

area is considerably smaller than that of either telescope beam (1,500 arc min² for the MK 1A telescope at this frequency), independent interferometer beams could be directed simultaneously at the flare star, a phase reference source, and one or more regions of blank sky. This instrument thus gives excellent discrimination against both terrestrial emissions and confusion by the faint and numerous extragalactic radio sources.

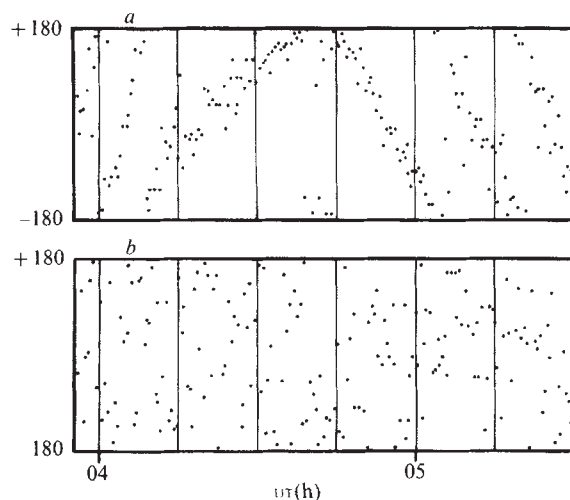
In a preliminary search a source of 220 mJy at

RA 07 h 42 min 22 s $\pm$ 1s Dec 03°30' $\pm$ 1' (1950)

was found to be suitable for use as a phase reference. (This source seemed to be double with a component separation of 13 arc s in P.A. 45° and is probably extragalactic.) When this source was used as a phase reference the random noise level could be reduced by integration, and the system gave unambiguous (3σ) detection of any flares more intense than 38 mJy with duration >15 min. In 48 h of observation of the star YZ CMi (Ross 882; RA 07h42min04s Dec+03°41' (1950)) in the period 12–18 December 1977 flare emission greater than this limit was detected for a total of 5 h from two separate flares. The data on these flares are given in Table 1.

Figure 1 shows 60-min Fourier transforms; *a*, near the peak of the more intense flare; *b*, on a blank sky channel at the same sidereal time; *c*, on the flare star channel on a different date; and *d*, on the channel directed at the reference source at the same sidereal time. The response for the flare has its theoretical width, which shows that ionospheric irregularities were not degrading these integrations. This flare was sufficiently intense to be observed by inspection of the phase plots on the 30-s on-line record. The interferometer used has an output phase rate of one turn (360°) per hour for a position offset of approximately 5 arc s, though ionospheric effects may introduce phase gradients of this order. The absolute phase rate observed (Fig. 2*a*) shows that the source of this emission was within 15 arc s of the position of YZ CMi, thus providing additional confirmation that the emission came from that star.

Fig. 2 The raw phase plots of the output channel directed at the star. *a*, During the maximum of the flare of 18 December 1977. *b*, At the same UT on 14 December 1977 (a non-flare epoch).



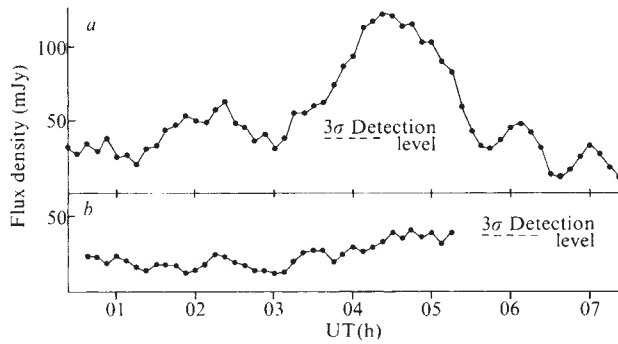


Fig. 3 Three-point running means of $7\frac{1}{2}$ min integrations on the star channel during the two flares on YZ CMi. *a*, 18 December; *b*, 15 December 1977.

Figure 3 shows the variation of the emission with time for both flares. The more intense flare showed at least two peaks of emission. The emission from the weaker flare was only found after 15-point (7.5 min) integrations had been carried out: its amplitude was still increasing when the observations ceased. Integrations lasting 5 h were also carried out to search for continuous emission from YZ CMi outside the flare epochs. Combination of these integrations on five nights did not reveal any correlated emission, and enabled an upper limit of ≤ 4 mJy to be set to any continuous radio flux from the star.

Table 1 Data on two radio flares from YZ CMi

Date	Flare onset (UT)	Duration (h)	Max intensity (mJy)
1977			
Dec 15	0330	1	≥ 41
Dec 18	0130	4	120

The energy of the 18 December flare over a 4 MHz band centred on 408 MHz was 2.8×10^{18} J. This may be compared with the energy of 1.5×10^{20} J over a similar band measured during a flare lasting 3 h observed on YZ CMi on 19 January 1969 (ref. 5). A large solar flare (importance 3) emits radio energies of order 1.2×10^{14} J in one hour in the same band centred on 408 MHz. Thus, the present measurements confirm that the energies of even the weak radio flares emitted by a red dwarf star are several orders of magnitude greater than those from large and infrequent solar outbursts.

The signal-to-noise ratio obtained during these observations was at least 10 to 1 after 1 h integrations. This makes it possible, for the first time, to obtain unambiguous detections of weak flares of long duration from radio observations alone. It is anticipated that further use of these techniques, at various radio frequencies and in combination with optical observations, will lead to progress in understanding the processes occurring in the atmospheres of the red dwarf flare stars.

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1. Lovell, B., Whipple, F. L. & Solomon, L. H. *Nature*, **198**, 288 (1963).
2. Snee, O. B., Solomon, L. H. & Patson, G. E. *Nature*, **199**, 991 (1963).
3. Lovell, B., Mavridis, L. N. & Contadakis, M. E. *Nature*, **250**, 124 (1974).
4. Spangler, S. R. & Moffett, T. J. *Astrophys. J.*, **203**, 497 (1976).
5. Lovell, B. *Nature*, **222**, 1126 (1969).
6. Peckham, R. J. *Mon. Not. R. astr. Soc.*, **165**, 25 (1973).

Upper limits to X-ray emission from colliding stellar winds

STELLAR winds are an important form of mass loss for many stars. In a binary system containing two similar mass losing stars, such winds can interact and shock to produce X rays¹⁻³. The spectrum and intensity of radiation emitted by the shocked gas gives information on the velocity and rate of mass loss. Here we discuss a range of candidate systems in which such processes may be occurring.

We estimate the expected X-ray luminosity from the collision of winds from two identical stars. We take the mass loss from each star to be $10^{-6} M_{\odot} \text{ yr}^{-1}$, the velocity of the wind to be $10^8 v_8 \text{ km s}^{-1}$ and the stellar separation to be $10^{13} a_{13} \text{ cm}$. For this separation the binary period is $17 a_{13}^{3/2} (M/40 M_{\odot})^{-1/2} \text{ d}$, where M is the total binary mass. We assume a circular orbit. To obtain an optimistic estimate of the luminosity we assume initially that the cooling time t_{cool} for the shocked material is much less than the flow timescale t_{flow} (or relevant timescale for adiabatic expansion). If t_{cool} is much greater than t_{flow} , the estimated luminosity should be reduced by a factor $t_{\text{cool}}/t_{\text{flow}}$.

The post shock temperature of the wind is $T_s = 1.5 \times 10^7 v_8^2 \text{ K}$ and the post shock density, assuming the shock to occur at a distance of $a/2$ from each stellar centre, is $n_s = 5.0 \times 10^9 M_{\odot} v_8^{-1} a_{13}^{-2} \text{ cm}^{-3}$. For such a gas the bremsstrahlung cooling timescale $t_b = 1.6 \times 10^5 M_{\odot}^{-1} v_8^2 a_{13}^{-2} \text{ s}$. The flow timescale $t_{\text{flow}} \sim c_s^{-1} a/2$ where c_s is the sound speed in the shocked gas. We find $t_{\text{flow}} = 1.3 \times 10^5 v_8^{-1} a_{13} \text{ s}$. The expected luminosity from the shocked region is then equal to $L_1 = f M \dot{v}^2$ where f is a geometrical factor (the fraction of the wind shocked to $\sim T_s$) which we estimate to be equal to about 0.1. Thus

$$L_1 = 7 \times 10^{34} \dot{M}_{-6} v_8^2 \text{ erg s}^{-1} \quad (1)$$

If bremsstrahlung cooling is relevant and if $t_b \gg t_{\text{flow}}$, that is, if $\dot{M}_{-6} v_8^{-3} a_{13}^{-1} \ll 0.8$, the luminosity is

$$L_2 = 8 \times 10^{34} \dot{M}_{-6} v_8^{-1} a_{13}^{-1} \text{ erg s}^{-1} \quad (2)$$

We selected several close binary systems for observation in which both stars are thought to be O-stars undergoing mass loss (Table 1). The 3σ upper limits to the 2–10-keV X-ray flux from these systems have been obtained from the Ariel 5 Sky Survey data (see ref. 4). None of these systems was positively detected by Ariel 5. We have converted the flux upper limits to upper limits to the X-ray luminosity, L_0 , using the distances shown in Table 1. For comparison we have tabulated the simple X-ray luminosity, L_e , estimated from equations (1) or (2), the minimum of (L_1, L_2) being appropriate. These estimates are in units of $\dot{M}_{-6} v_8^{-1} a_{13}^{-1} (M/40 M_{\odot})^{-1/3} \text{ erg s}^{-1}$ for equations (1) and (2), respectively. The upper limits are comparable to, or less than, the predicted values for typical wind parameters; $\dot{M} \sim 10^{-6} M_{\odot} \text{ yr}^{-1}$, $v \sim 1,000 \text{ km s}^{-1}$.

We also obtained an upper limit of $\sim 10^{32} \text{ erg s}^{-1}$ on the Wolf-Rayet binary, γ^2 Velorum, which consists of a WC8 and a O9I star¹¹. Mass loss is so strong in this system¹², however,

Table 1 Close binary systems

Name	Period (d)	Spectral type	Distance (kpc)	Min $L_{1,2}$ ($\times 10^{34}$)	L_0 ($\times 10^{34}$)	Ref.
HD47129	14.4	O7+?	1	7.0	<4.3	5
HD75759	33.3	O9V+O9V	1.5	5.1	<5.6	6
HD93205	6.08	O3V+O8V	2.6	7.0	<49	7
HD159176	3.3	O7+O7	1	7.0	<2.2	8
HD199579	48.6	O6+?	~ 1	4.0	<4.3	9
HD206267	3.7	O6+?	~ 1	7.0	<8.0	10

that the electron scattering optical depth is ≥ 1 . Any X-ray radiation is probably degraded in energy or absorbed before leaving the system, and thus the constraint provided by the above upper limit is weak. X-ray absorption is important at kilovolt energies when $M_{-6}/a_{13}v_8 \geq 0.1$.

In this way, we see that constraints can be obtained on the stellar wind parameters. For each particular system, however, the actual constraints deduced in this way may be complicated by other considerations. First, if the pre-shock velocity $v_8 \leq 1$, then most of the emitted flux emerges as soft X rays below the pass band of the Ariel 5 detectors. Observations at soft X-ray energies, for example, by HEAO-1, HEAO-B and other satellites, could be used to check this. Second, in some systems, Compton cooling of the shocked gas by ultraviolet stellar photons may compete with bremsstrahlung cooling. Third, the geometrical collision factor, f , which we have taken to be 0.1 may be smaller if one wind is substantially stronger than the other.

We now consider another situation in which colliding winds might produce X-ray emission. The stellar birth process often produces close associations of O stars. We suggest that the Trapezium stars in the Orion Nebula are responsible for the X-ray source 4U0531-05/2A0532-056 (refs 13, 14). The distance to the nebula is 460 pc which implies that the average separation of these stars is $a \sim 3 \times 10^{16}$ cm. We estimate the geometrical collision factor for four such similar stars to be ~ 0.5 , yielding from equation (2) $L_2 \approx 8 \times 10^{31} \dot{M}_{-6}^2 v_8^{-1} a_{16.5}^{-1} \text{ erg s}^{-1}$. The observed flux of $\sim 3 \times 10^{-13} \text{ erg s}^{-1}$ requires $\dot{M} \sim 6 \times 10^{-6} M_{\odot} \text{ yr}^{-1}$ from each star. X-radiation may also emerge where the stellar winds interact with the surrounding nebula.

Mass loss from later type stars could also generate soft X rays. For example, Capella, which is a binary system containing a G5III and a G0III star¹⁷, has already been detected in soft X rays^{15,16} with a soft X-ray luminosity of $\sim 2 \times 10^{29} \text{ erg s}^{-1}$. For such stars we expect $v_8 \sim 0.3$, $T_s \sim 2 \times 10^6 \text{ K}$ and hence that line cooling predominates in the shocked gas. Taking this into account we find that a mass loss rate of $\sim 3 \times 10^{-10} M_{\odot} \text{ yr}^{-1}$ would be needed from each star to account for the observed soft X-ray flux.

We conclude by noting that current X-ray observations already place constraints on the velocity and mass loss rate of stellar winds in O-star binaries. Future soft X-ray observations of these objects, of systems such as Capella, and in particular imaging observations of the Orion Nebula, will be important as sources of information on the density and velocity structure of stellar winds.

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1. Prilutskii, O. F. & Usov, V. V. *Sov. Astr.* **20**, 2-4 (1976).
2. Cherepashchuk, A. M. *Sov. Astr. Lett.* **2**, 138-139 (1976).
3. Wallerstein, G. *Astrophys. J.* **151**, L121-L123 (1968).
4. Cooke *et al.* *Mon. Not. R. astr. Soc.* **182**, 489-516 (1978).
5. Hutchings, J. B. & Cowley, A. P. *Astrophys. J.* **206**, 490-498 (1976).
6. Thackeray, A. D. *Mon. Not. R. astr. Soc.* **134**, 97-106 (1966).
7. Conti, P. S. & Walborn, N. R. *Astrophys. J.* **207**, 502-505 (1976).
8. Conti, P. S., Cowley, A. P. & Johnson, G. B. *Publ. astr. Soc. Pac.* **87**, 327-332 (1975).
9. Batten, A. *Publ. Dom. astr. Obs.* **13**, 119-251 (1967).
10. Crampton, D. & Redman, R. O. *Astr. J.* **80**, 327 (1975).

11. Conti, P. S. & Smith, L. F. *Astrophys. J.* **172**, 623-630 (1972).
12. Seaquist, E. R. *Astrophys. J. Lett.* **203**, L35-L37 (1976).
13. den Boggende, T. *et al. Astr. Astrophys.* **62**, 1-7 (1978).
14. White, G. J. & Ricketts, M. J. *Astrophys. Lett.* **18**, 79-81 (1977).
15. Catura, R. C., Acton, L. W. & Johnson, H. M. *Astrophys. J. Lett.* **196**, L47-L49 (1975).
16. Mewe *et al. Astrophys. J. Lett.* **202**, L67-L71 (1975).
17. Wright, K. O. *Publ. Dom. astr. Obs.* **10**, 1-37 (1954).

Resonance region of a PC5 micropulsation examined by a dual auroral radar system

SINCE January 1977 a new radar auroral backscatter facility, STARE—Scandinavian Twin Auroral Radar Experiment—has been in operation in northern Scandinavia. It consists of two multibeam radars that are sensitive to electrostatic ion waves (irregularities) produced in association with current in the auroral zone E region. The radars measure the intensity and Doppler velocity of irregularities within a 200,000 km² common area. The measurements are made with an effective spatial resolution of approximately 20 × 20 km² and a temporal resolution of 20 s. From the Doppler velocity data it is possible to determine the mean irregularity drift velocity field within the common area. It has been shown¹ that this mean velocity is approximately equal to the electron drift velocity which, in the E region, is given by $\mathbf{E} \times \mathbf{B} / B^2$. Because of the spatial and temporal resolution of the STARE system, it is well suited for the study of ionospheric electric fields associated with PC5 geomagnetic pulsations (period 150-600 s). Here we present the first results of the analysis of such an event which occurred between 1230 and 1300 UT (~ 1500 -1530 MLT) on 2 February 1977. This event is typical of one class of PC5 events that we are studying. The magnetic data used in the study come from one of a chain of high precision rubidium vapour magnetometers designed for geomagnetic pulsation research. These have been operated in Scandinavia since October 1976 by the Institute of Geological Sciences as part of its participation in the IMS. These instruments have an essentially flat frequency response and a noise level typically ≤ 0.1 nT. The magnetic variations in three orthogonal directions (magnetic NE, NW, and Z) are sampled at 2.5-s intervals with a daytime resolution of 0.08 nT and digitally recorded on magnetic tape cassettes.

Figure 1 shows an example of a drift velocity pattern observed by STARE within the viewing area during the event. This pattern represents a 20-s average and values are plotted at 20-km intervals in geographic longitude and latitude. The precise method of deriving this plot is described by Greenwald *et al.*². Each dot represents a location where the drift velocity vector has been determined. The magnitude and direction of the velocity is represented by the associated line.

As this plot can also be viewed as the electron drift velocity pattern in regions where irregularities exist, the electric field map can also be obtained. This is done by rotating the vectors 90° clockwise and setting $1,000 \text{ m s}^{-1} \approx 50 \text{ mV m}^{-1}$.

In the auroral E-region the Hall current is carried predominantly by the electrons and flows in the opposite direction to their drift. The Pedersen current is carried exclusively by the ions. If the Hall and Pedersen conductivities are spatially uniform and constant during the event, then the Hall current is proportional to the electron velocity. Therefore, Fig. 1 can give us a picture of (1) the electron drift velocity; (2) the electric field, and (3) the Hall current in the ionosphere.

The drift pattern in Fig. 1 consists of alternating bands of predominantly eastwards and westwards motion. These bands drift polewards through the viewing area as can be seen more clearly in Fig. 2, which plots the average velocity in the longitude sector between 16.0° and 18.0° as a function of latitude and time. Figure 2 clearly shows the alternating bands of eastwards and westwards drift and their polewards movement. A similar polewards drift of intensity enhancements in radar

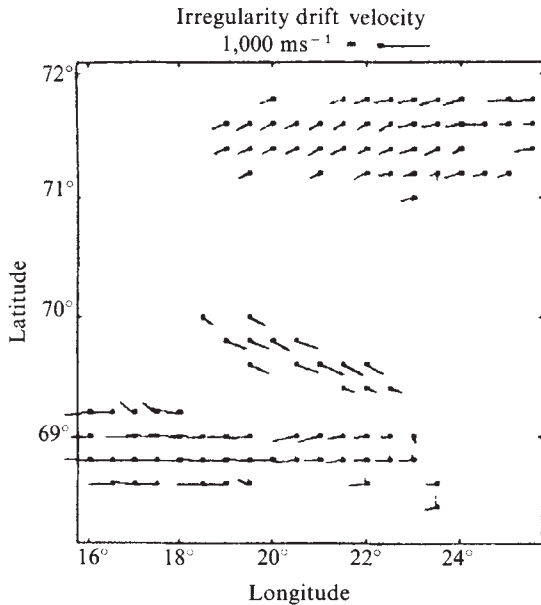


Fig. 1 The drift velocity pattern of E-region irregularities at day 33 1977 1244 UT during the event. The spots represent the positions at which the velocities have been measured. The lines represent the velocity vectors. The electron velocity is the same as the irregularity velocity. The electric field is proportional to the velocity and its direction is found by rotating the vectors 90° clockwise.

auroral backscatter has previously been reported^{3,4}. These enhancements are also observed in the STARE data, but they will not be discussed here.

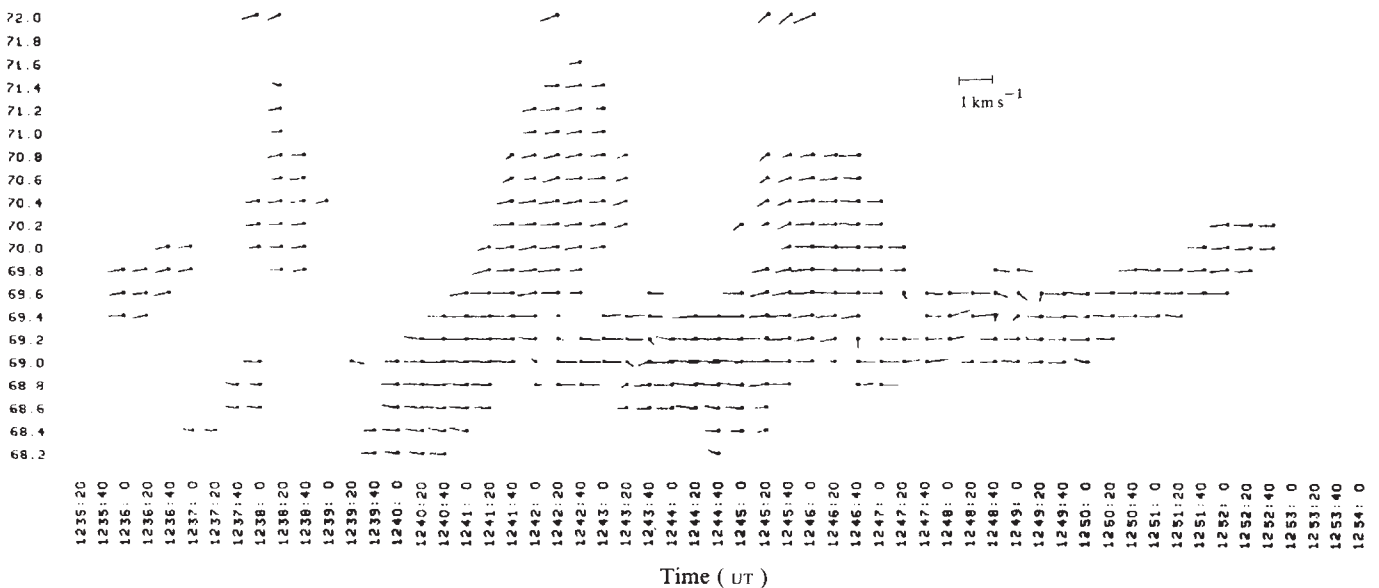
Recent theories have shown that the magnetic field components observed on the ground in association with long period pulsations are rotated through 90° from their values above the ionosphere⁵⁻⁷. In essence, if the ionospheric conductivity is uniform, the magnetic field associated with field-aligned currents (that is, the part having a curl parallel to the Earth's magnetic field) is screened from the ground by ionospheric Pedersen currents. The field observed on the ground in this case arises from the associated Hall currents. This

suggests that the field on the ground can be calculated from the observed Hall currents by using the Biot-Savart law.

Using the data from plots similar to Fig. 1, we have calculated the expected magnetic field perturbations for Tromsø, Norway (69° 39' N, 18° 56' E). The Biot-Savart integration was carried out over a 300 km square grid centred over Tromsø. The currents outside this area have been ignored. We have estimated the effect of this exclusion to be a 25% reduction in the magnitude of the predicted fields on the ground. Their temporal behaviour should be unaffected. We have also neglected the effects of induced currents in this calculation. We estimate that for a conductivity of $10^{-2} \Omega^{-1} \text{m}^{-1}$ the H and Z components are unaffected by the ground because of the short scale length of the horizontal variation. If the D component has a much larger scale length the Earth may increase its predicted value by a factor of up to 2. Its phase might also be affected. The predicted H , Z and D perturbations are compared with the Tromsø magnetic data in Fig. 3. The predicted magnetic perturbations have been normalised by assuming an E-layer density of $1.5 \times 10^{11} \text{ m}^{-3}$ and a nominal Hall current sheet thickness of 20 km. Both of these are reasonable values and they yield very good agreement between the two data sets. In particular, we observe remarkably good agreement between the predicted and observed H and Z components in both frequency and phase. Furthermore, the same normalisation has yielded good agreement in amplitude for these components. The predicted and observed D components show less agreement in both phase and frequency.

We suggest the following explanation for these results: Southwood⁸ and Chen and Hasegawa⁹ assume a surface wave on the magnetosphere boundary (for example, a wave driven by the Kelvin-Helmholtz-instability as a result of solar wind motion). This wave is evanescent within the magnetosphere. The scale length of the evanescence is comparable with the wavelength of the surface wave and, hence, it penetrates well into the magnetosphere. The oscillation within the magnetosphere has components perpendicular to and in the meridian plane because of coupling between toroidal and poloidal oscillations. Normally, the toroidal oscillation is forced and has an amplitude comparable with the poloidal oscillation. However, near a field line with a natural frequency equal to that of the oscillation, there is resonance and the amplitude of the toroidal motion is quite large. In the afternoon the magnetospheric disturbance maps into the ionosphere as an eastwards propagating wave with estimated wavelength of the order of several thousand kilometres. At most latitudes the wave has north-south and east-west components of electric field that are below the level necessary to produce the instabilities giving rise to radar auroral irregularities. However, over the narrow band of

Fig. 2 Mean velocity vectors in the longitude sector 16–18.0° as a function of geographic latitude and time (UT), on day 33 1977.



latitudes where the field lines resonate in the toroidal mode, the north-south electric field is very large, instabilities are produced, and we can measure the electric field structure. The latitudinal scale length of this resonance region seems to be about 250 km in the ionosphere.

The good prediction (using the Biot-Savart law) of the H and Z components on the ground and the failure to predict D successfully can be explained as follows: immediately below the ionosphere the H , D , and Z disturbances can be represented by two-dimensional Fourier integrals of the form

$$H = \iint G(k_x, k_y) \exp[-i(k_x x + k_y y)] dk_x dk_y$$

where x is northward and y eastward.

The angular spectrum functions of H and Z are strongly peaked at values of k_x near $k_{x0} = 2\pi/l$, where l is a length, characteristic of the width of the resonance region (about 250 km). At the same time they will have peak values of k_y near $k_{y0} = 2\pi/\lambda$, where λ is the wavelength in the east-west direction (several thousand kilometres). The largest contribution to the integral comes from values of k_x and k_y near k_{x0} and k_{y0} . The angular spectrum function for D , on the other hand is not peaked for values of k_x near $2\pi/l$, but it does have the peak near k_{y0} . The largest contribution to this integral comes from components with k_y near k_{y0} and with values of k_x characteristic, not of the width of the resonance region, but of the much greater distance corresponding to the depth of penetration into the magnetosphere mapped down to the ionosphere. Below the ionosphere each Fourier component varies as

$$\exp[-i(k_x x + k_y y + k_z z)]$$

where

$$k_x^2 + k_y^2 + k_z^2 = \omega^2/c^2$$

For these frequencies, $\omega/c \ll k_x, k_y$ and thus

$$k_z \approx \pm i(k_x^2 + k_y^2)^{1/2}$$

so that the waves decay evanescently downwards as $\exp(-k_z z)$.

In all cases the scale length of the evanescence is approximately the same as the horizontal scale length of the Fourier component. The Fourier components making the major contribution to H and Z have values of k_x near k_{x0} ($\gg k_{y0}$). They are therefore reduced in amplitude at the ground by a factor of the order of $\exp(-2\pi h/l)$ (about 0.06 for $h \approx 110$ km). The components making the major contribution to D , on the other hand, are reduced in amplitude by a much smaller amount (about 0.5 if we set both λ and the x scale length to about 1,000 km). The observation that D , H , and Z have comparable amplitudes on the ground implies that, on the resonant field line, in the ionosphere and magnetosphere, the associated wave electric and magnetic fields differ by a factor of the order of 10. This behaviour is shown by the results of computations of Hughes and Southwood¹⁰. Hence, in the E-region, the east-west electric field is appreciably below threshold for the radar auroral instabilities. We, therefore expect that D will not be well predicted by the STARE observations. If this interpretation is correct then a ground-based measurement of the polarisation ellipticity will not give a value closely related to the magnetospheric value. The polarisation ellipse in the magnetosphere is substantially more elongated.

There are many other features of the theories of Southwood⁸ and Chen and Hasegawa⁹ which can be studied by STARE. For example, we have found that the predicted 180° phase change of the north-south electric field across the resonance region explains the poleward motion of the scattering regions seen earlier^{3,4}. The theory predicts that the apparent polewards motion of the irregularities is consistent with an eastwards propagating wave on the magnetopause. Such detailed checks of the theory are too elaborate for presentation here and will form part of a more detailed analysis of the observations.

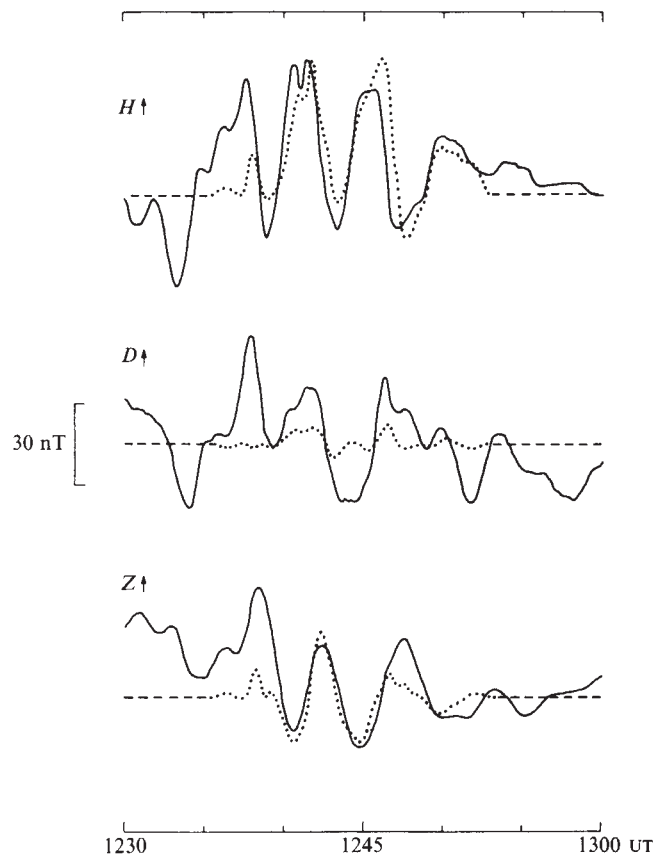


Fig. 3 Comparison between the predicted behaviour of magnetic field components on the ground and actual magnetograms. Magnetometer data (solid line); fields predicted by Biot-Savart law (dotted line); disturbance below threshold for excitation of radar irregularities (dashed line).

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1. Ecklund, W. L., Balsley, B. B. & Carter, D. A. *J. geophys. Res.* **82**, 195-197 (1977).
2. Greenwald, R. A., Weiss, W., Nielsen, E. & Thomson, N. R. *Radio Sci.* (in the press).
3. Kaneda, E., Kokubun, S., Oguti, T. & Nagata, T. *Rep. Ionosph. Space Res. Japan*, **18**, 165-172 (1964).

4. McDiarmid, D. R. & McNamara, A. G. *Ann. Geophys.* **28**, 433–441 (1972).
5. Nishida, A. *J. geophys. Res.* **69**, 1861–1874 (1964).
6. Inoue, Y. *J. geophys. Res.* **78**, 2959–2976 (1973).
7. Hughes, W. J. *Planet. Space Sci.* **22**, 1157–1172 (1974).
8. Southwood, D. J. *Planet. Space Sci.* **22**, 483–491 (1974).
9. Chen, L. & Hasegawa, A. *J. geophys. Res.* **79**, 1024–1032 (1974).
10. Hughes, W. J. & Southwood, D. J. *J. geophys. Res.* **81**, 3241–3247 (1976).

Electric fields in the ionosphere produced by polar field-aligned currents

TWO regions of field-aligned currents of magnetospheric origin have recently been found^{1–3} along the auroral oval and region 1 currents flow at a slightly higher latitude than those in region 2. Figure 1 shows the distribution and flow directions of such field-aligned currents determined from Triad data⁴. We discuss here the effects that these currents seem to have on the distribution of electric fields in the ionosphere, in addition to the S_a electric field of ionospheric origin.

We have solved numerically the equation $\text{div } \mathbf{J} = -j_{\parallel}$, making some simplifying assumptions; the left-hand side shows a two-dimensional divergence of the height-integrated horizontal current, $\mathbf{J}(\mathbf{J} = \Sigma \cdot \mathbf{E}$, where $\mathbf{J}_x$ = southwards component, $\mathbf{J}_y$ = eastwards component), on the ionospheric shell, and the right-

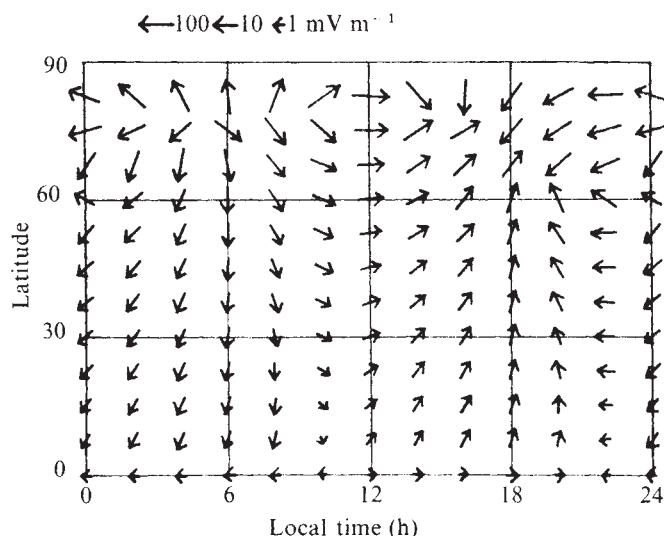


Fig. 2 Distribution of electric fields due to the field-aligned currents for Case 1 where $j_{\parallel}(2) = (1/8)j_{\parallel}(1)$.

that the distribution of $j_{\parallel}(1)$ and $j_{\parallel}(2)$ has a pattern similar to that shown in Fig. 1 and a sinusoidal change with longitude along the oval (the amplitude of $j_{\parallel}(1)$ is $2.0(\mu\text{A m}^{-2})$, and that the distribution of anisotropic conductivities, Σ_{xx} ($=9.0 \times 10^{-9}$ e.m.u.), Σ_{yy} ($=7.0 \times 10^{-9}$ e.m.u.) and Σ_{xy} ($=2.0 \times 10^{-8}$ e.m.u.), is uniform over the latitude and longitude (or local time). It is seen that the electric field at the Equator has an amplitude of about 1.4 mV m^{-1} , an eastwards direction from 1100 to 2100 LT and a westwards direction from 2200 to 1000 LT.

Figure 3 shows the distribution of electric fields calculated for case 2 where $j_{\parallel}(2) = (1/2)j_{\parallel}(1)$, on the same assumption as in Fig. 2. It is seen that the electric field at the Equator has an amplitude of about 1.7 mV m^{-1} , a westwards direction from 1100 to 2300 LT and an eastwards direction from 2400 to 1000 LT. Figures 2 and 3 show that the distributions of the electric field in the polar cap are similar, because the electric field in the polar cap is mainly determined by a fairly steady value of $j_{\parallel}(1)$.

Fig. 3 Distribution of electric fields due to the field-aligned currents for Case 2 where $j_{\parallel}(2) = (1/2)j_{\parallel}(1)$.

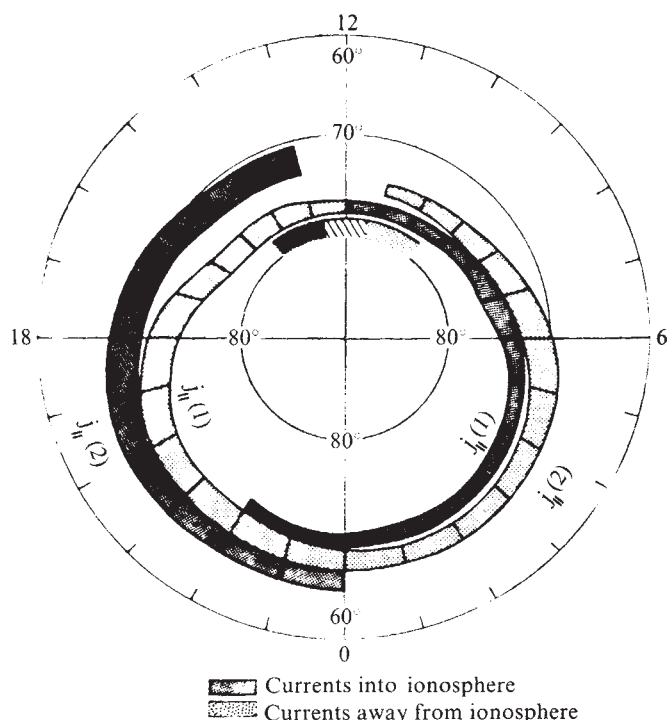
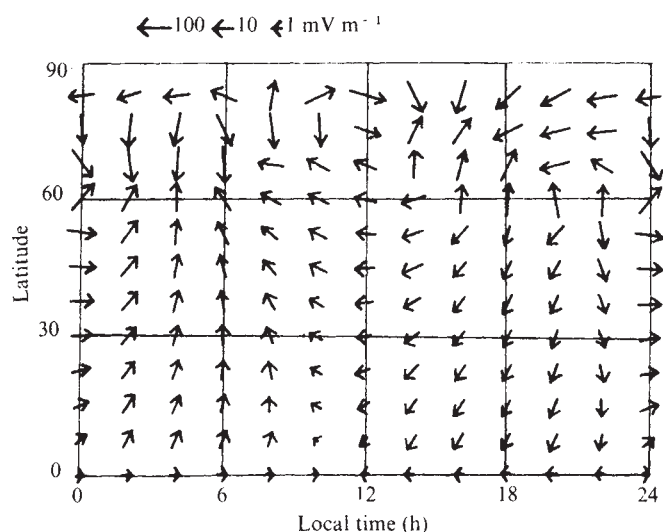


Fig. 1 Distribution and flow directions of polar field-aligned currents determined from Triad data⁴.

hand side shows field-aligned currents in region 1 (denoted by $j_{\parallel}(1)$) and region 2 ($j_{\parallel}(2)$). At middle and low latitudes the magnitude of the electric fields is found to be several mV m^{-1} , and their gross pattern in the latitude-time plane is counter-clockwise (clockwise) during the day-time (night-time) when the ratio $j_{\parallel}(2)/j_{\parallel}(1)$ is small; it is clockwise (counter-clockwise) when the ratio is large.

Figure 2 shows the global distribution of these electric fields calculated for case 1 where $j_{\parallel}(2) = (1/8)j_{\parallel}(1)$. Here we assume

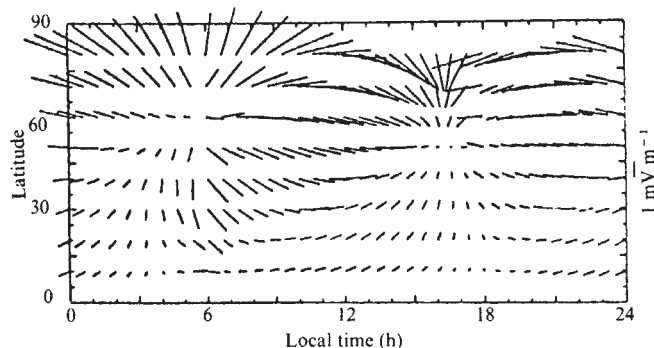


Fig. 4 Distribution of polarisation electric fields derived from the ionospheric-wind dynamo theory⁵.

Figure 4 shows the distribution of polarisation electric fields derived from the ionospheric-wind dynamo theory⁵. Comparing this with Figs. 2 and 3, the electric field of magnetospheric origin is found to be of the same order of magnitude as that of ionospheric origin at middle and low latitudes. This strongly suggests that the electric field of ionospheric origin at middle and low latitudes is influenced by field-aligned currents in the polar region to the extent that the distribution of electric fields is sometimes reversed at middle latitudes. In this case we may expect anomalous behaviour in S_q currents and electron drifts at middle latitudes, and also in the electrojet, the sporadic E layer and Appleton anomalies of the F layer at the Equator. A fuller account will be published elsewhere.

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1. Zmuda, A. J. & Armstrong, J. C. *J. geophys. Res.* **79**, 4611-4619 (1974).
2. Sugiura, M. & Potemra, T. A. *J. geophys. Res.* **81**, 2155-2164 (1976).
3. Iijima, T. & Potemra, T. A. *J. geophys. Res.* **81**, 2165-2174 (1976).
4. Iijima, T. & Potemra, T. A. *J. geophys. Res.* **81**, 5971-5979 (1976).
5. Matsushita, S. *Radio Sci.* **4**, 771-780 (1969).

Transmission of polar electric fields to the Equator

SEVERAL kinds of geomagnetic variations are observed simultaneously at high latitudes and in the equatorial region. These are substorms or DP-1 variations, S_q^p or DP-2 variations, some kinds of pulsations, and the DS-part of SSC and SI. Araki¹ analysed the relationship of the preliminary reverse impulse of SC* occurring in the high-latitude and the equatorial regions and found that their occurrence in both regions is well correlated, almost simultaneous, and that the waveforms are very similar. From the characteristics of these geomagnetic variations, it seems that a horizontal electric field impressed in the polar ionosphere from the magnetosphere greatly contributes to the equatorial part of these geomagnetic variations. This electric field is generated by large scale dynamic processes

in the magnetosphere such as a sudden compression by an interplanetary shock wave or a change of the magnetospheric convection, and its direction will be approximately east-west (dawn-dusk)². To clarify the mechanism of the instantaneous horizontal transmission of the polar electric field, we have analysed the response of the ionosphere to a suddenly impressed electric field.

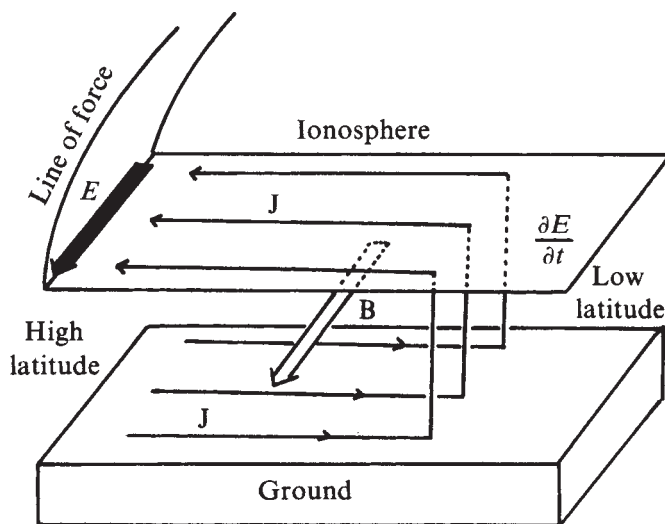
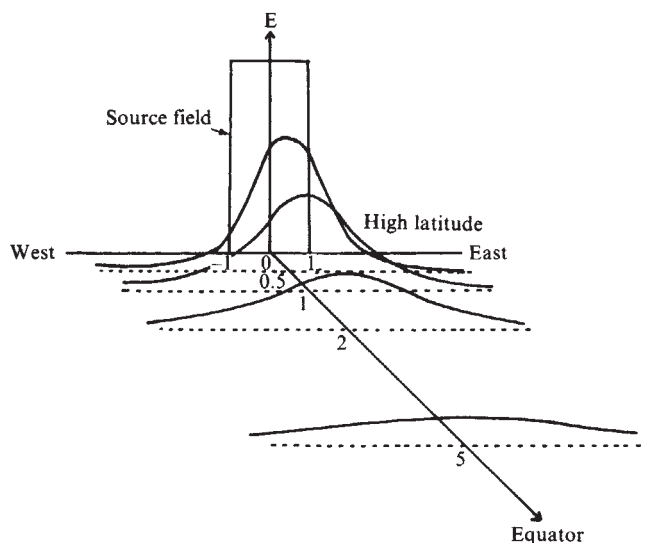


Fig. 1 Horizontal propagation of electric fields impressed on the high-latitude ionosphere.

If we consider that an electromagnetic disturbance propagates in the unbounded uniform ionosphere (E-region), the equation describing its propagation is of the diffusion type, and the propagation velocity is several km s^{-1} (refs 3, 4). In this case the polar electric field takes more than several tens of minutes to propagate to the Equator⁵. However, if we solve the two-dimensional initial and boundary value problem of plane geometry, the vacuum region below the ionosphere is found to play an important role for the instantaneous transmission of the polar electric field. As shown in Fig. 1, the east-west electric field communicated along the geomagnetic field lines to high latitudes induces a north-south Hall current in the polar iono-

Fig. 2 Attenuation of electric fields due to finite scale of a source field. Distance in the north-south direction is normalised by the east-west scale of the source field.



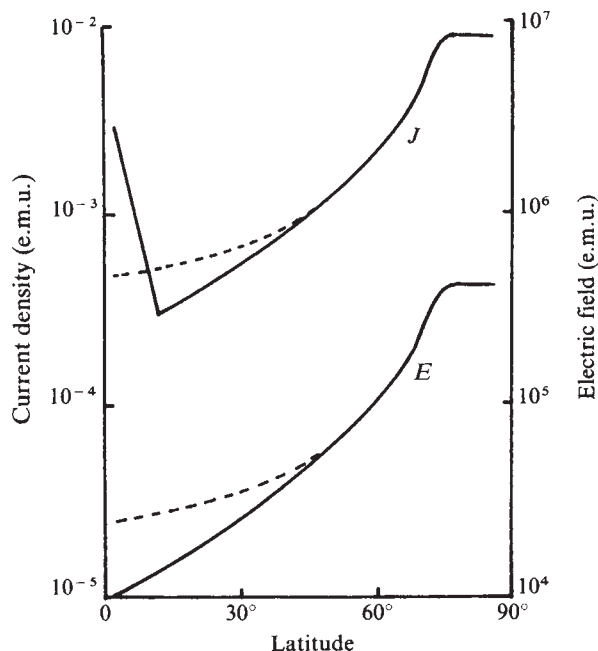


Fig. 3 Latitudinal variation of electric fields, E , and currents, J , at noon (1200 h, solid line) and midnight (0000 h, dashed line). The ionospheric conductivity is assumed to be isotropic and uniform at all latitudes on the night-time and at latitudes greater than 10° on the day-time. In the day-time equatorial region, it varies sinusoidally with local time and is maximum (10.5 times the uniform value) at the noon equator.

sphere and a current of reverse sense in the ground. These two currents are connected by a vertical displacement current, $\partial E/\partial t$, which flows on the horizontally propagating wavefront. The resultant current loop makes an east-west magnetic field, B , in the vacuum waveguide bounded by the ionosphere and the Earth's surface. This east-west magnetic field can propagate instantaneously in the north-south direction, as the zero-order transverse magnetic (TM_0) waveguide mode⁵, accompanying the east-west electric field in the ionosphere.

If the east-west scale of the source field in the high latitude region is finite, the propagating field suffers geometrical attenuation as shown in Fig. 2 where the ionospheric conductivity is assumed to be uniform, and it is analytically estimated that the amplitude of the equatorial electric field becomes about 1/10 of the polar electric field for the scale of DP-2 type variations.

To estimate the attenuation more accurately, we have solved⁶ the continuity equation of the electric current by assuming a current source on the 75° lat. circle, and obtained the global distribution of electric fields and currents. Figure 3 shows a result of this calculation for an isotropic ionospheric conductivity which is enhanced at the daytime Equator. The electric field decreases monotonically with decreasing latitude, but the electric current increases due to the conductivity enhancement at the daytime equator and makes a significant contribution to geomagnetic variations there.

Although we have calculated the electric field and current for a simple model, we conclude that the equatorial ionosphere is well connected to the polar ionosphere by the electric field. Thus, an electric field originating in the magnetosphere at high L -values communicates to the polar ionosphere along the geomagnetic field lines, and then propagates instantaneously to the Equator, resulting in geomagnetic variations similar to those in the polar region. As the TM_0 mode has no cut-off frequency, electric fields of any period can propagate to the Equator.

The electric field thus propagated to lower latitudes can be transferred to the magnetosphere at low L -values along the field lines and may have some significant effects.

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1. Araki, T. *Planet. Space Sci.* **25**, 373-384 (1977).
2. Tamao, T. *J. geophys. Res.* **80**, 4230-42 (1975).
3. Jacobs, J. A., Rostoker, G. & Watanabe, T. *Nature* **205**, 61-62 (1965).
4. Rostoker, G. *J. geophys. Res.* **70**, 4388-4390 (1965).
5. Kikuchi, T. & Araki, T. (To be published.)
6. Maekawa, K. & Maeda, H. *Nature* **273**, 649-650 (1978).

Threshold effects in the excitation of autoionising states of argon atoms by electron impact

ATOMIC autoionising states lying above the first ionisation limit of the atom decay by electron ejection and can be excited using several techniques involving photon, ion and electron impact. Here we present measurements made using low-energy electron impact and show clearly the power of this method for investigating triplet states that are difficult to populate by more conventional techniques. Observations were also made of the striking effects of the post-collision interaction between the scattered electron that has excited the autoionising state and the subsequently ejected electron. This effect is significant when the states are excited close to threshold and results in spectral features showing a sharp onset followed by broad oscillations rather than a normal line shape. The onset is significantly narrower than the natural width of the state responsible and we propose here that this is due to a selection mechanism on its decay time.

Autoionising states were excited using a monochromatic beam of electrons and the energy spectrum of scattered electrons was measured. The electron spectrometer used was part of a spectrometer described previously¹. It consisted of a conventional electron energy selector with hemispherical deflectors to produce the monochromatic electron beam which was crossed with the target beam. The scattered electrons were detected using a similar hemispherical analyser with a channel multiplier detector.

In this study special attention was focused on the problem of energy calibration. Fortunately, it was possible to obtain a reliable and accurate calibration using a weak double-scattering process which is observable in this energy range. The most prominent feature in the electron energy-loss spectrum of argon is due to the $3p^5 4s(P)$ state at 11.828 eV. Electrons which have excited this state twice in consecutive collisions with two atoms give a peak in the energy-loss spectrum at 23.65 eV and this can be used as a calibration point as the energy of the state involved is well established by photo-absorption spectroscopy². Any features due to double scattering can readily be distinguished from the single-scattering features by their quadratic pressure dependence. It has been established by this means that none of the other structure

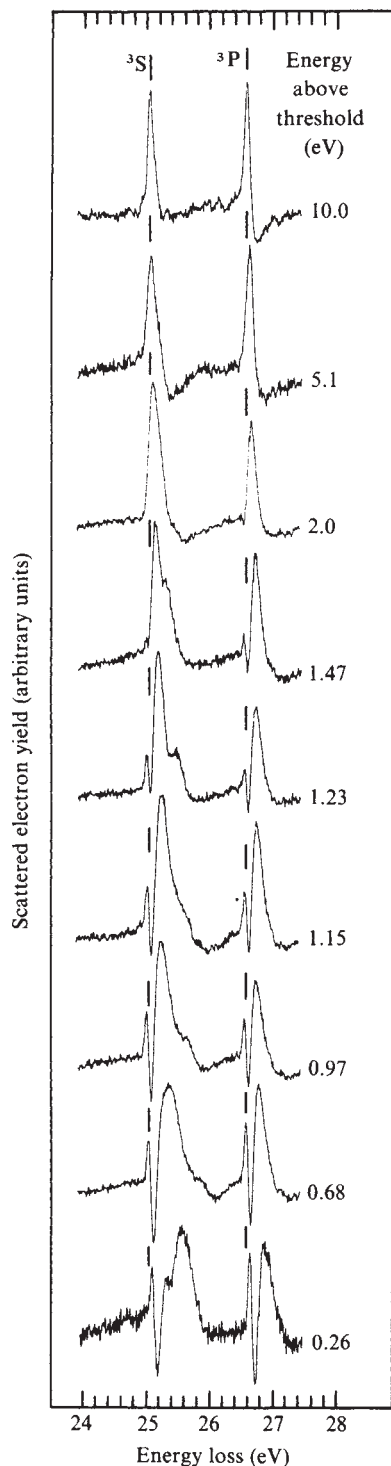


Fig. 1 Electron energy-loss spectra measured in argon. Each spectrum was obtained with a constant energy for the collected electrons and the excess energy above the threshold for exciting the autoionising states is given in eV. The measured energies of the $3s3p^6 4s$ (3S) and $3s3p^6 4p$ (3P) states are indicated by vertical dashes. All spectra were taken at an electron scattering angle of 0° relative to the incident beam with the exception of the 5.1 eV spectrum which was obtained at 40° .

subsequently presented here is due to double scattering. Even the most prominent calibration peak is very weak on all our spectra and in most cases it was necessary to increase the pressure in order to observe it clearly.

Figure 1 shows energy-loss spectra measured in argon. On each spectrum both the impact energy and energy loss are

scanned in order to maintain a constant excess energy above the threshold for exciting the states involved. At 10 eV excess energy above threshold two narrow lines are observed. These are due to the excitation of autoionising states and in the absence of any complications the spectra measured at different excess energies should show structure having a simple symmetric or asymmetric Beutler-Fano resonance profile, the resonance centre being at a well defined energy. This is clearly not generally true for either of the structures observed in Fig. 1. As the energy above threshold is lowered the structure splits into oscillations which become more pronounced and extend to higher energy loss as the impact energy approaches the threshold for exciting these states. This phenomenon can be attributed to a Coulomb interaction between the scattered electron, the ejected electron subsequently produced in the decay of the autoionising state and the remaining positive ion. This interaction is significant because the scattered electron is very slow ($6 \times 10^5 \text{ m s}^{-1}$ at 1 eV) and the autoionising state is very short-lived (typically 10^{-15} s) so that the scattered electron is close to the atom when the ejection occurs. This effect, which has become known as the post-collision interaction (PCI) was first observed in electron impact spectra by Hicks *et al.*³. A classical analysis of the process⁴ predicts a profile whose centre shifts to higher energy as the energy above threshold decreases. However, a quantal or semi-quantal treatment^{5,6} is able to explain the presence of oscillations in the cross section and the present data represent the clearest evidence for these which has yet been seen.

Before further discussion of PCI effects in argon it is useful to consider the origin of the original structure. The lowest state of Ar^+ is a doublet $3p^5(^2P_{3/2})$ at about 15.8 eV and between these levels there are several autoionising series which converge on the higher level. Above this energy the lowest autoionising states are the $3s3p^6 4s$ (3S) and the $3s3p^6 4p$ (3P) near 25.0 and 26.6 eV which are formed by the promotion of a 3s electron. At still higher energies there are many singly and doubly excited states^{7,8} but the only ones which can reasonably be expected in the energy region covered by the spectra in Fig. 1 are the 1S and 1P states noted above.

The width of the 1S state is known to be about 130 meV (refs 9, 10) and its energy is 25.23 eV (refs 9–11). The prominent feature in the 10-eV spectrum where post-collision interaction effects are expected to be small occurs at 25.03 eV and has a measured width (FWHM) of 110 meV. The measured width of the apparatus function (55 meV) can then be used to deduce¹² the natural width of the state and gives a value of 80 meV. This feature can therefore only be due to the 3S state which lies 0.2 eV lower than the 1S state. This splitting is similar to that of the analogous states in magnesium, where the $3s4s(^3S)$ lies 0.29 eV below the $3s4s(^1S)$. This indicates that the $3s3p^6 4s(^3S)$ state in argon which is observable at high impact energies¹¹ is very weak at 10 eV above threshold, although it may be responsible for some of the structure observed at or above the 1S energy on other spectra.

The $3s3p^6 4p(^1P)$ state has been studied extensively and is observed at an energy of 26.606 eV with a natural width of $80 \pm 5 \text{ meV}$ in photoabsorption⁷. On the spectrum taken at 10 eV above threshold a slightly asymmetric structure is observed, the peak being at 26.56 eV. The observed width of the profile is 82 meV giving a natural width of 40 meV (ref. 12). This is too narrow to be due to the 1P and must therefore be the $3s3p^6 4p(^3P)$ state. This state has previously only been reported by Fryar and McConkey⁸ and by Veillette and Marchand¹³ who obtained a value of 26.41 eV. These energies disagree with our value. Fryar and McConkey identified the structure on spectra measured close to threshold where the centre of the structure is shifted by PCI effects. They then extrapolated to obtain the spectroscopic energy of the state and this may account for the discrepancy. Veillette and Marchand¹³ measured the broadband photon yield from argon as a function of incident electron energy and as this is effectively a threshold measurement the results may also be severely affected by PCI and by the

presence of negative ion states in this region. This point has been discussed by Wilden *et al.*¹⁴. The fact that our measured width is small means that PCI effects are not important at 10 eV above threshold and we therefore believe that our value for the energy is reliable. Comparison with the analogous 3s4p(³P) state in magnesium indicates that there is no significant broadening due to the splitting of the triplet sub-levels. The information derived about the states in this region is summarised in Table 1.

One particular feature of the data presented here is the very sharp onsets which are observed in the two lowest-energy spectra. The initial peak of the ³S oscillation on these two spectra has an observed width (FWHM) of 55 meV and, as the apparatus function has a measured width of 55 meV, it is clear that this initial peak is much narrower than the natural width (80 meV) of the ³S state. The atoms which are excited to the ³S state have a distribution of decay times and this very sharp onset could be interpreted as being due to a selection mechanism in which the fraction of the ³S states which are long-lived give scattered electrons which are not significantly shifted in energy by the post-collision interaction. These therefore appear close in energy to the spectroscopic threshold for the state with a very sharp onset which reflects the long decay time of the atoms responsible for this part of the structure.

Table 1 Energies *E* and widths Γ of triplet states in argon (in eV)

Level	Present results		Previous measurement of <i>E</i>		
	$\Gamma(\pm 0.02)$	<i>E</i> (± 0.03)	Ref. 8 (± 0.03)	Ref. 13	Ref. 15
3s3p ⁶ 4s(³ S)	0.08	25.03	25.02	25.02	24.96
3s3p ⁶ 4p(³ P)	0.04	26.56	26.46	26.41	

The detailed structure observed in the region of the ³S state is clearly more complicated than that due to the ³P state. In particular, a broad shoulder is observed to the high energy side of the main peak in the ³S structure on all the spectra below 2 eV excess energy. In addition, sharper structure is observed close to the ¹S energy on the spectrum taken at 0.26 eV above threshold. These effects may be due to the ¹S state and would be expected to produce structure shifted to even higher energy.

As a result of this study, two of the low-lying autoionising states in argon, 3s3p⁶4s(³S) and 3s3p⁶4p(³P), have been positively identified and their widths and energies measured. Very clear evidence of oscillations due to the post-collision interaction has been observed together with a selection mechanism on the lifetime of the autoionising states which gives an onset significantly sharper than the natural widths of the states involved.

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- Wilden, D. G., Hicks, P. J. & Comer, J. *J. Phys. B* **9**, 1959 (1976).
- Moore, C. E., Atomic Energy Levels *NBS Circ. No. 467* (Printing Office, Washington DC US Govt, 1971).
- Hicks, P. J., Cvejanović, S., Comer, J., Read, F. H. & Sharp, J. M. *Vacuum* **24**, 573 (1973).
- Barker, R. B. & Berry, H. W. *Phys. Rev.* **151**, 14 (1966).
- Read, F. H. *J. Phys. B* **10**, L207 (1977).
- Morgenstern, R., Nighaus, A. & Thielmann, U. *Phys. Rev. Lett.* **37**, 199 (1976).
- Madden, R. P., Ederer, D. L. & Codling, K. *Phys. Rev.* **177**, 136 (1969).

- Fryar, J. & McConkey, J. W. *J. Phys. B* **9**, 619 (1976).
- Hicks, P. J., Sharp, J. M., Comer, J. & Read, F. H. *Proc. 8th Int. Conf. Physics of Electronic and Atomic Collisions*, Belgrade Abstr. 515 (Institute of Physics, Belgrade, 1973).
- Hicks, P. J., Sharp, J. M. & Comer, J. (in preparation).
- Bergmark, T. *et al. Uppsala Univ. Inst. Phys. Rept. No. 589* (1969).
- Comer, J. & Read, F. H. *J. Electron. Spectrosc.* **2**, 87 (1973).
- Veillette, P. & Marchand, P. *Can. J. Phys.* **52**, 930 (1974).
- Wilden, D. G., Hicks, P. J. & Comer, J. *J. Phys. B* **10**, 1477 (1977).
- Bolduc, E., Quemener, J. J. & Marmet, P. *Can. J. Phys.* **49**, 3095 (1971).

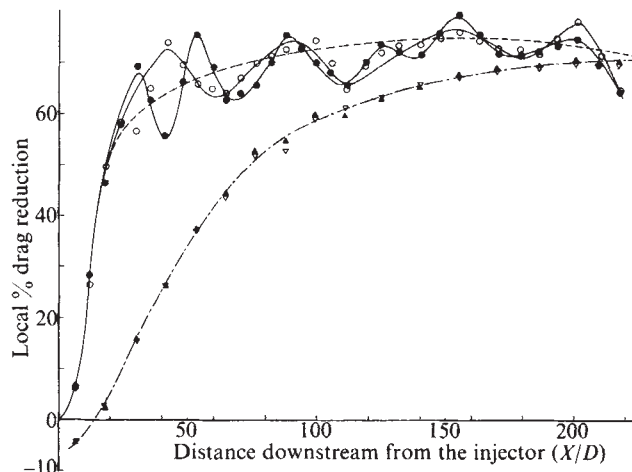
Drag-reducing polymers and turbulent bursts

THE flow in a pipe is said to become well developed at the point where the axial pressure gradient ceases to depend on the distance along the tube. At such a point, the injection of drag-reducing polymer solutions produces a developing situation in which the magnitude of the observed drag reduction increases in the streamwise direction. This variation is due to the polymers spreading out radially as they are carried downstream. It continues until the polymer is uniformly distributed over the cross-section of the pipe. We have found that when the injection is at the pipe wall, the resulting streamwise variation of wall friction can have an oscillatory character, and we report here that this may be related to the phenomenon of turbulent bursts¹⁻⁴.

Our experiments were carried out on a turbulent flow of water in a horizontal pipe of circular section. To ensure that the flow was well developed, the injection point was preceded by an entrance length. We injected relatively concentrated solutions of polyacrylamide (Separan AP 30) or polyethyleneoxide (WSR 301) at either the wall or the centre-line. Downstream from the injection point a series of pressure taps monitored the streamwise variation of wall friction. Thus, the developing situation could be characterised by a local percentage drag reduction, defined at the midpoint of any pair of adjacent pressure taps. Measurements were also made of the radial dispersion of the polymer, using a sampling system. Laser anemometry was used to assess the effect on the turbulent structure.

Figure 1 shows typical results for injection at the centre-line and at the wall (two runs in both cases). These results show that polymer injection at the wall has a more rapid effect than that at the centre. This is consistent with the usual picture of the polymer acting in the buffer region of the flow. The slight

Fig. 1 Variation of local drag reduction with distance downstream from the polymer injection point. An aqueous solution of Polyox WSR 301, at a concentration of 3,000 w.p.m., was injected into a water flow at sufficient rate to give a bulk mean concentration of 10.8 w.p.m. Injection at centre line, Δ , run 1; injection at centre line, ∇ , run 2; injection at wall, $\bullet$, run 1; injection at wall, $\circ$, run 2. In all cases, the Reynolds number, $Re = 4.5 \times 10^4$.



'overshoot' in the mean (dotted line) of the wall-injection data is also consistent with this view.

However, it is the oscillation of the data points for wall injection that concerns us here. At first this was thought to be random scatter but after inspecting many sets of data, it seemed clear that this was a quasi-cyclic process (as defined by Offen and Kline²).

Two possible explanations were considered. First, there could be some structural instability in the (injection) flow due to the non-newtonian nature of the fluid being injected. Such instabilities did occur occasionally during our experiments. But they were much smaller than this effect, and tended to occur only at very low injection flow rates. Second, the turbulent bursting process could be modulating the outward diffusion of the polymer from the wall. This was supported by the general resemblance between the oscillatory pressure curves (for example, Fig. 1) and the velocity signal found during the bursting process^{1,2}. Thus, the second idea seemed more likely and was tested as follows.

Consider the oscillatory part of the drag reduction curves in Fig. 1. Let the distance between successive crests be L_x (that is, the analogue of the wavelength for a regular periodic function, but L_x is a random variable). If the oscillations are due to turbulent bursts, the mean value of L_x should be related to T_B , the mean time between bursts. Thus $T_B = \langle L_x \rangle / U_B$ where U_B is the streamwise mean velocity of the large-scale burst structures and $\langle \rangle$ denotes mean value.

We evaluated $\langle L_x \rangle$ from three or four cycles of each pressure curve (as in Fig. 1) and from seven such curves in all. The velocity U_B was taken to be $0.8U_0$, where U_0 is the centre-line mean velocity⁵. The result was $T_B = 0.41 \pm 0.06$ s (as predicted from drag reduction curves).

To check this, we used the laser anemometer to measure the autocorrelation of the streamwise fluctuating velocity, $R_{11}(\tau)$. Individual (single-realisation) values of T_B were obtained from the first cycle of the $R_{11}(\tau)$ curve¹. Then 49 such curves were used to form an ensemble average and hence $T_B = 0.43 \pm 0.05$ s, for the mean time between bursts.

The agreement between the two values of T_B suggests that the oscillations in Fig. 1 are evidence of interaction between the injected polymer solution and the turbulent bursts. This effect may be particularly relevant to the view that the polymers reduce drag by stabilising the wall layer with a consequent reduction in bursting^{6,7}.

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1. Kim, H. T., Kline, S. J. & Reynolds, W. C. *J. Fluid Mech.* **50**, 133 (1971).
2. Offen, G. R. & Kline, S. J. *J. Fluid Mech.* **70**, 209 (1975).
3. Ueda, H. & Hinze, J. O. *J. Fluid Mech.* **67**, 125 (1975).
4. Antonia, R. A., Danh, H. Q. & Prabhu, A. *Phys. Fluids* **19**, 1680 (1976).
5. Brown, G. L. & Thomas, A. S. W. *Phys. Fluids* **20**, S243 (1977).
6. Gadd, G. E. *Nature* **206**, 463 (1965).
7. Achia, B. U. & Thompson, D. W. *J. Fluid Mech.* **81**, 439 (1977).

Investigating artificially coloured diamonds

COLOURLESS diamonds are rare—most have a slight natural yellow colour which makes them unsuitable as gem stones. However, a strongly coloured gem quality diamond is very valuable. Neutron irradiation is sometimes used to 'treat' diamonds to strengthen and improve the yellow colour, but their value is decreased if the coloration is known to be artificial. For many years gem testing laboratories have concentrated on an absorption line at 595 nm to decide the

pedigree of yellow coloured diamonds. Crowningshield¹ examined a very large number of treated and untreated diamonds and found that all treated diamonds except one showed the 595 nm line, whereas in only two cases of uncertain history was this line present in natural untreated stones. The rarity of this line in untreated diamonds has been confirmed by Anderson² who further states (personal communication) that it is never seen in a cut and polished sample. The apparent universality of the 595 nm line in treated diamonds, and its absence in untreated diamonds has therefore been used as a guide to a diamond's history, and indeed the Gemological Institute of America has based its certificates of authenticity on this criterion¹. This note draws attention to the fact that absorption at 595 nm in a treated diamond may be destroyed without significantly altering the colour of the stone.

Colourless type IA diamonds (diamonds that contain nitrogen in predominantly aggregated forms) may be given a golden amber colour by radiation damage and subsequent annealing. After the irradiation the diamond has a blue or green colour due to absorption in the GR1 band which has a zero-phonon line at 741 nm (ref. 3). (The colour centres described here are all vibronic systems which have a sharp zero-phonon line at the long wavelength limit, and a band of overlapping broader peaks at shorter wavelengths. Although most of the absorption occurs in this band, the colour centres will be described by the wavelength of the zero-phonon line (measured at ~ 77 K).) Heating the diamond to about 700–800 °C destroys the GR1 band and results in the formation of the H3 and H4 centres which have zero-phonon lines at 503 and 496 nm. The GR1 centre is believed to be the neutral vacancy⁴ which becomes mobile at high temperatures and wanders through the crystal until it is trapped at one of the forms of nitrogen (or recombines). The most important forms of nitrogen in type IA diamond are the A-aggregate and the B-aggregate, and the concentrations of these two aggregates can be determined from the impurity-induced one-phonon infrared absorption between 7 and 10 μ m (ref. 5). Davies⁶ has shown that the ratio of the H3 and H4 absorption produced in irradiated and annealed diamonds is the same as the ratio of the concentrations of A and B nitrogen.

Most diamonds have a natural yellow colour due to the so-called Cape absorption series with prominent lines at 415 and 478 nm. In the majority of cases the diamonds are only slightly coloured and their value is less than that of a 'pure white' stone. A strongly coloured diamond (a so-called 'fancy' yellow specimen) may, however, be extremely valuable because of its rarity.

The yellow colour imparted to a diamond by radiation damage and annealing appears similar to the eye to the yellow colour associated with the naturally occurring Cape absorption lines. There is, therefore, considerable opportunity for financial gain if a poorly coloured diamond is improved (treated) by irradiation and annealing, and the practice has been growing in the past 20 years. Neutron irradiation is generally used as this rapidly produces uniform radiation damage throughout the whole crystal. Naturally, if it is known that a yellow diamond owes its colour to treatment its value as a gemstone is dramatically reduced and it is important, therefore, to establish reliable tests to investigate this possibility. At first sight such a test may seem trivial as spectroscopic examination would rapidly indicate the presence of H3 and H4 absorption. Unfortunately, none of the absorption bands in irradiated diamonds is entirely unknown in natural diamonds², and the H3 system is frequently observed in brown or greenish untreated diamonds.

I have recently investigated the annealing behaviour at high temperatures of optical centres in irradiated diamonds. In Fig. 1 the strengths of the GR1, H3 and 595 nm zero-phonon lines are shown as a function of the annealing temperature. We note that at 700–800 °C, the temperature region normally used for colouring diamonds, the GR1 line has virtually disappeared and the H3 and 595 nm lines have reached their maximum intensity. However, if the annealing temperature is increased to

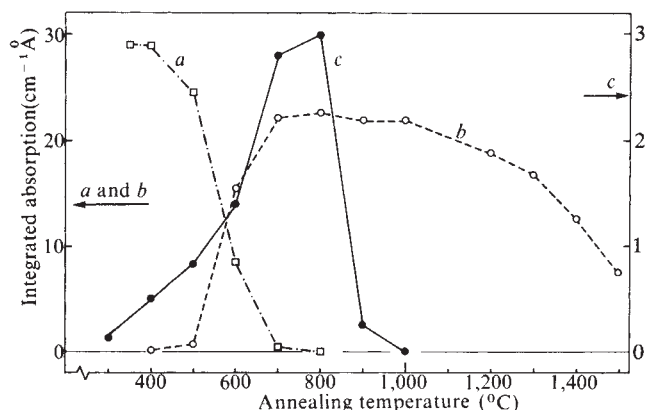


Fig. 1 Integrated absorption (absorption coefficient in $\text{cm}^{-1} \times$ full width at half height in $\text{\AA}$) of *a*, GR1, 741 nm; *b*, H3, 503 nm; and *c*, 595 nm zero-phonon lines, measured at 77 K after the diamond had been annealed at the temperatures shown. For temperatures up to 1,200 °C the annealing time was 12 h at each point; above 1,200 °C, 5 h at each point. The diamond, originally colourless, had been irradiated with $\sim 3 \times 10^{21}$ 2 MeV electrons m^{-2} . Left-hand axis refers to (*a*) and (*b*), right-hand axis to (*c*).

1,000 °C the 595 nm line completely disappears, whereas the H3 absorption controlling the colour of the diamond is not significantly changed. (This result has been confirmed by M. H. Nazaré (personal communication).) Only at higher temperatures does the H3 centre itself begin to anneal out.

Clearly, the absence of absorption at 595 nm does not unambiguously categorise a diamond as untreated, and other criteria must be adopted. A further clue comes from the relative strengths of the H3 and H4 absorption bands. Anderson⁷ points out that in nature the H3 line is the more prominent member, whereas in treated stones H4 is of equal or even greater prominence. Thomaz and Davies⁸ have also noted that H3 centres which are present naturally in diamonds are often not accompanied by H4 centres, regardless of the ratio of *A* to *B* nitrogen. The concentration of *B* nitrogen is often relatively high in diamonds showing strong Cape absorption⁶. We therefore expect the H4 band to be prominent (in most cases) when a Cape yellow diamond has been treated to improve its colour, as observed experimentally⁷. It is not clear at present why the H4 to H3 ratio is lower than the *B* to *A* nitrogen ratio in untreated specimens, but clearly this helps to detect artificially coloured diamonds. Ideally, one should compare the H3 to H4 ratio with the *A* to *B* nitrogen ratio; where these are equal the diamond has almost certainly been treated, even if there is no detectable absorption at 595 nm. Unfortunately the *A* and *B* nitrogen concentrations cannot be determined by conventional infrared absorption measurements in most gem-sized diamonds because the absorption coefficient between 7 and 10 μm is too large. Alternative techniques are therefore required before it can be decided with absolute certainty whether diamonds in which the 595 nm band is absent have been artificially coloured.

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1. Crowningshield, G. R. *Gems Gemol.* 9, 99–104 (1958).
2. Anderson, B. W. *J. Gemmol.* 9, 1–11 (1963).
3. Clark, C. D., Ditchburn, R. W. & Dyer, H. B. *Proc. R. Soc. A* 234, 363–381 (1956).
4. Walker, J., Vermeulen, L. A. & Clark, C. D. *Proc. R. Soc. A* 341, 253–266 (1974).
5. Davies, G. *Chem. Phys. Carbon* 31, 1–143 (1977).
6. Davies, G. & Summersgill, I. *Diamond Research* 1973 6–15 (Suppl. Ind. Diamond Rev. 1973).
7. Anderson, B. W. in *Gem Testing* (Butterworths, Sevenoaks, 1971).
8. Thomaz, M. F. & Davies, G. *Proc. R. Soc.* (in the press).

What happened to the high-latitude palaeomagnetic poles?

MANY apparent polar paths for the Precambrian have been published recently. A striking feature of these paths, besides their spaghetti-like appearance, is their bias for the equatorial zone, or their apparent aversion to high latitudes^{1–3}. Does this mean that contrary to the present pole, the ancient pole had a much greater affinity for the equatorial region? Perhaps high latitude poles do not exist, or they are not reported, or they are discriminated against in construction of polar paths. From data given here it seems that only the Pleistocene, the late Palaeozoic⁴ and Aphebian glacial deposits^{5,6} were truly formed in high latitudes. We discuss here whether some other mechanism is needed to explain the implied low latitude of deposition of other glacial deposits, and whether all these other deposits were genuinely polar but the palaeomagnetic record, as presently known, is deficient in high latitude poles. Difficulty in finding high latitude poles could arise from one or more of the following causes: inadequate (and biased) sampling; tectonic effect; and interpretation of high latitude poles.

With the limited number of palaeomagnetic results available for certain periods of geological time, there can be little doubt the palaeomagnetic record is far from complete. In issues 1 and 2 of the Ottawa catalogues^{7,8} there are 433 palaeomagnetic poles reported from Precambrian rock units: this is a limited number of data points for a period as long as 3,000–4,000 Myr (an average of one pole per 7–9 Myr), especially considering that they were obtained from several continents (or land masses) that may have been in relative motions throughout that time. Does this incomplete record provide us with a bias, that is, do the missing gaps repetitiously hide a certain feature in the record? Only a complete record can answer this question. Until then we must hope that the deficiencies of the record do not produce a seriously biased data set so that, incomplete as it may be, the available record may still be of some use. Here we shall consider the sampling unbiased and look into other causes for the apparently missing high latitude poles.

Only 29% of the Earth's surface occupies latitudes greater than 45°; only 13% lies in latitudes above 60°. If we assume that over geological time the distribution of poles was uniform over the surface of the Earth, then one determination out of seven should yield a high latitude (60°) pole. However, the ancient position of a pole will be accurately determined only if

Fig. 1 Distribution of Precambrian Shield area of the world. Associated with each, their relative ratio of observed over expected observations in percentage. The first value in parentheses represents the % ratio in the latitude interval 45–90° (north and south), the second, the interval 60–90°.

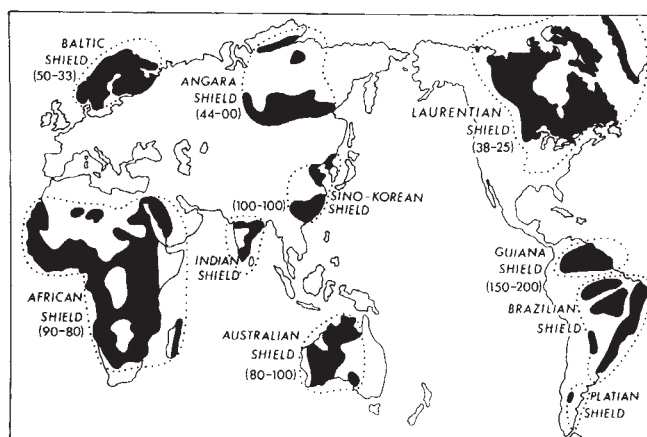


Table 1 High latitude world Precambrian palaeomagnetic poles distribution

Shield	Observed distribution						Expected distribution		Ratio (observed/expected)	
	N_{obs}^* 0–90°	%† wt	N_{obs} 45–90°	N_{obs} 60–90°	%‡ 45–90°	% 60–90°	$N_{\text{exp}}^{\S}$ 45–90°	N_{exp} 60–90°	% $R_{\parallel}$ 45–90°	% R 60–90°
Laurentian	268	61.8	30	9	11.1	3.3	79	36	38	25
Baltic	47	10.8	7	2	14.9	4.3	14	6	50	33
African	35	8.0	9	4	25.7	11.5	10	5	90	80
Angara	31	7.1	4	—	12.9	—	9	4	44	—
Indian and Sinokorean	25	5.7	7	3	28.0	12.0	7	3	100	100
Australian	17	3.9	4	2	23.5	11.8	5	2	80	100
Guiana, Brazilian and Platian	7	1.6	3	2	42.9	28.6	2	1	(150)	(200)
Arctic	2	0.5	0	0	—	—	1	0	—	—
Antarctic	1	0.2	0	0	—	—	0	0	—	—
World observations	433	100	64	22	5.0	14.7	127	57	50	39

* No. of observations in the latitudinal band specified, these observations are all A and B types defined ref. 8.

† Percentage of world total observations from each continent.

‡ Percentage of observations in the latitudinal band specified.

§ No. of observations expected for the specified latitudinal band.

|| $N_{\text{obs}}/N_{\text{exp}} \times 100$; percentage of observed over expected observations in the specified latitudinal band. Parentheses indicate a statistically unreliable value owing to the small sample population.

the land mass (or sampling area) can be relocated on the present grid system exactly in its latitudinal location and azimuthal orientation at the time of remanence acquisition. Any error in relocating the land mass to its exact original location and orientation will result in a surplus of low latitude poles. For example, if a palaeo-equator land mass which recorded a high latitude pole is incorrectly rotated, the pole will plot into lower latitudes. Of course, both low and high latitude poles could be artificially generated in this manner, but, because the high latitude area is so limited and such a great distance away from the sampling area, this azimuthal misorientation favours the lower latitude zone. This problem still exists but becomes less serious for a sampling area located in intermediate and high palaeolatitudes. Unfortunately, another problem becomes dominant. The curve expected from a geocentric axial dipole is not linear and the relation, $\tan$ of latitude = $1/2 \tan$ of inclination (ref. 9), makes it difficult to determine accurately a high latitude pole from a high latitude sampling area. For example, a latitude of 45° corresponds to an inclination of 63.5° and a latitude of 60° to an inclination of 74°. Therefore, any error in the determination of inclination could introduce an error twice as large in latitudinal positioning. The spherical shape of the Earth again dictates that this will favour lower rather than higher latitudes. It seems then that the relocation problem may be quite serious.

These error effects will increase the scatter of poles, not only in the present polar zone, but also along the ancient polar path. In the present geometry, this would be most visible as a lack of poles in the high latitude zone.

The distribution of observations from Precambrian rock units amongst continents or shields is shown in Fig. 1 and Table 1. A compilation of data¹⁰ shows that 384 palaeomagnetic poles have been reported from Lower Palaeozoic (Cambrian–Devonian) rocks. The distribution of observations amongst continents is given in Table 2. The number of poles found in the latitude intervals 45–90° (north and south) have been compiled for each continent. Assuming a uniform distribution of poles over the Earth's surface, the number of poles expected to be found in those intervals is also given; 29.3% and 13.4% of the total for the 45–90° and 60–90° intervals. Comparison of the % ratio (observed/expected) of high latitude poles reported from continents in temperate and polar latitudinal zones and from continents in the equatorial zone sets us an enigma. In the former the % ratio is never greater than 50% while in the latter the % ratio is near and sometimes even exceeds 100%.

For example, the number of high latitude (60–90°) poles for North America is four times less than expected. We then have two possible explanations: either pole paths genuinely adhere to equatorial zones meaning that our assumption of uniform

Table 2 High latitude world lower Palaeozoic palaeomagnetic poles distribution*

Continent	Observed distribution						Expected distribution		Ratio (observed/expected)	
	N_{obs} 0–90°	% wt	N_{obs} 45–90°	N_{obs} 60–90°	% 45–90°	% 60–90°	N_{exp} 45–90°	N_{exp} 60–90°	% R 45–90°	% R 60–90°
USSR	170	44.3	22	1	0.6	12.9	50	23	44	04
Europe	90	23.4	4	2	2.2	4.4	26	12	15	17
North America	41	10.7	4	0	—	9.8	12	5	33	—
Australia	35	9.1	18	13	37.1	51.4	10	5	180	260
South America	18	4.7	6	3	16.7	33.3	5	2	120	150
Asia	13	3.4	2	1	7.7	15.4	4	2	50	50
Africa	10	2.6	4	2	20.0	40.0	3	1	133	200
Antarctica	7	1.8	2	1	14.3	28.6	2	1	(100)	(100)
Arctica	0	—	0	0	—	—	—	—	—	—
World observations	384	100	62	23	16.1	5.9	112	51	55	45

* See Table 1 legend.

distribution is invalid, or high latitude poles have been incorrectly interpreted, not reported or ignored in polar path constructions. It may be that the approach to palaeomagnetic directions that approximate to the present Earth's field direction contributes substantially to the low % ratio. It is not uncommon to find, in palaeomagnetic studies of North American rocks, instances where such magnetisations have been arbitrarily attributed to recent remagnetisation. Little regard, however, has been paid to the mechanism by which this (parasitic?) remagnetisation was effected. It is difficult to explain why. Rock surfaces in the higher latitude continents were effectively scoured by Pleistocene glaciers and it is expected that remagnetisation would be rare. Strict examination of the field and laboratory stability evidence rarely delimits the age of such magnetisations precisely. Why then is preference given to the present field? It is equally important to prove that such high latitude poles are attributable to recent remagnetisations, as genuine ancient poles. Neither the former, nor the latter should be blindly accepted.

Conversely the continents presently located in equatorial latitudes have a different situation. The percentage ratio is near 100, as expected. We are again faced with two possibilities: Either our assumption of uniform distribution of poles is valid or, by comparison with continents in higher latitudes, the number of high latitude poles is too high. Could it be that some recent remagnetisations have been mistaken for ancient magnetisations? Rock surfaces in tropical regimes are commonly covered by deep lateritic weathering profiles. Such weathering often develops remanence carriers of high thermal and alternating field stability, which could be incorrectly associated with polar antiquity¹¹.

This analysis presents us with an ironical situation. Studies from the higher latitude continents provide us with a much lower percentage of high latitude poles than studies from the equatorial continents.

In conclusion, our assumption of uniform distribution of poles may not be valid or there may be a sampling bias. Nonetheless, we feel the difference in % ratio between equatorial and higher latitude continents is so marked that it must have some physical significance. Either workers studying rocks from temperate and high latitudinal zones accept too easily the recent remagnetisation hypothesis and/or the workers studying rocks from equatorial zone are not easily swayed by this hypothesis. Clearly a more objective appraisal of the available stability evidence is required. Because of these difficulties in obtaining high latitude poles, the practice of casually attributing a high latitude pole to recent remagnetisation cannot be accepted. If the remagnetisation hypothesis is to be invoked evidence should be provided to support this interpretation¹².

This is Earth Physics Branch contribution 727.

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- Irving, E. & Lapointe, P. L. *Geosci. Can.* 2, 90 (1974).
- Irving, E. & McGlynn, G. C. *Philos. Trans. R. Soc.* 280, 433 (1976).
- Piper, J. D. A. *Nature* 251, 381 (1974).
- Irving, E. *J. geophys. Res.* 71, 6025 (1966).
- Roy, J. L. & Lapointe, P. L. *Can. J. Earth Sci.* 6, 749 (1976).
- Morris, W. A. *Geology* 5, 137 (1977).
- Hicken, A., Irving, E., Law, L. K. & Hastie, J. *Catalogue of Palaeomagnetic Directions and Poles* 1, 45 (1972).
- Irving, E. & Hastie, J. *Catalogue of Palaeomagnetic Directions and Poles* 2, 3 (1975).
- Creer, K. M. *J. geophys. Res.* 67, 3461 (1962).
- Irving, E., Tanczyk, E. & Hastie, J. *Catalogue of Palaeomagnetic Directions and Poles* 3, 5 (1976).
- Creer, K. M. *Nature* 219, 246 (1968).
- Roy, J. L. & Robertson, W. A. *J. geophys. Res.* 83, 1289 (1978).

Mercury in the Greenland Ice Sheet

REPORTS of the mercury content of the Greenland Ice Cap have varied within one or two orders of magnitude. Weiss *et al.*¹ found an increase from about 50 ng per kg in ancient deposit layers to about 100 ng per kg in surface samples from 1964 to 1965, and concluded that there was an increase in man's global input of mercury to the atmosphere. Their deposit and surface samples were collected in the Camp Century area (77° N, 61° W, Fig. 1) in north-west Greenland. Dickson² pointed out that this finding was due to a systematic difference between sampling sites and was supported by Carr and Wilkniess³, who in samples from Camp Century found 13 ng per kg and 50 ng per kg in deposits from 1850 and 1940, and in samples taken from site 2 (77° N, 56° W), about 150 km off Camp Century, found 169 ng per kg and 100 ng per kg in deposits dated 1870 and 1930. In contrast to their previous finding, Weiss *et al.*⁴ later found that the mercury concentration in seasonal samples of 1966–70, was similar to that of old deposits dating back to 1807 on 25 samples collected at Dye 3 station (65° N, 44° W). The average mercury concentration was 50 ng per kg of water. Herron *et al.*⁵ found the highest mercury concentrations yet reported from the Greenland Ice Cap ranging from 263 ng per kg to 881 ng per kg, covering ancient deposits from 1631 to surface pit samples in layers from 1972 to 1973. The 11 samples were collected at station Milcent (70° N, 45° W), and no significant increase in the mercury concentration was seen in modern times. The actual mercury level was taken as more representative of the Ice Cap than that previously found due to a new pretreatment of sample bottles. This controversy has stressed that far more information about the general level of mercury and more reliable sampling and analytical methods at ultra low mercury levels are needed. We have collected ice core samples at two sites, Dye 3 and Crete, which show a mercury level of about 10 ng per kg, in the order of magnitude of unpolluted ocean water. This level is one to two orders of magnitude lower than previous figures. We report here that the mercury contents of the Crete core show no increased values either in recent deposits or in deposits of 1783, the year of the volcanic eruption of Laki in Iceland.

Dye 3 (65° N, 44° W) and Crete (71° N, 37° W) cores were recovered under the Greenland Ice Sheet Program, and stored in the freezing room at the Geophysical Isotope Laboratory (GIL), Copenhagen. Eleven yearly mean samples were taken from the Crete core cover deposits from the period 1913–71, and a series of 14 annual mean samples over 1772–86. Samples originating from Dye 3 represent half-year deposits from 1902 and 1905, and a single year mean sample taken in deposits from 1727. Distinction between yearly deposits and dating of the layers is based on seasonal variation of the stable oxygen isotope ratio and microparticle concentration according to Johnsen *et al.*⁶ and Hammer⁷.

The samples were taken from GIL's freezing room and prepared using two different methods. (1) The plane surface of a half-cylinder of the ice core, corresponding to one-year (or half-year) deposits, was scraped by razor blade and scalpel, removing about 10 mm of the ice surface. The scraped surface was further cleaned by melting using a U-shaped graphite filled quartz-glass heater, developed by GIL for dust analysis sampling. A furrow was melted in the cleaned surface, and 10–30 ml meltwater (5–10°C) was pipetted into a 35 ml glass and covered with a PVC lid. Immediately afterwards, the sample was taken to a clean-room, and after a few minutes at room temperature the water was transferred to 3 or 10 ml quartz ampoules by pipetting. The ampoules were sealed by fusion while the sample was cooled in ice water. Samples of 1727, and 1902–38 were recovered by this method.

The upper porous firn deposits (1957–71) had to be sampled by another technique, because of the porosity of the ice. The

same technique was also used for samples of the series 1772–86, apart from additional cleansing by surface melting. (2) A 50 × 50 mm rod of ice was cut by band saw of the central part of the ice core. About 20 mm of the surface was removed with razor blade and scalpel. The cleaned rod, corresponding to a one-year deposit, was sectioned by cutting into a 75-ml glass and covered with a PVC lid. In the clean room (20°C) the sample was pipetted into 10 ml quartz ampoules or directly sucked into evacuated ampoules through a PVC tube and sealed. All glassware used for the sampling was precleaned in aqua regia and deionised water and dried.

The analysis for mercury in the sealed ampoules was carried out at the Danish Isotope Centre (DIC) using neutron activation analysis according to Sjöstrand⁸ with modifications. The method is based on simultaneous neutron activation of samples and standards, followed by chemical separation. After neutron irradiation (at a slow neutron flux of $5 \times 10^{12} \text{ n cm}^{-2} \text{ s}^{-1}$ for 48 h at the reactor in Institutt For Atomenergie, Kjeller, Norway) the ampoules are crushed in a closed vial in the presence of inactive mercury carrier and digestion agent, transferred to a Bethge distillation apparatus, and digested. Mercury is separated from other elements by distillation and electrolytic deposition on gold foils.

The radioactive mercury content was determined in a NaI-detector measuring the γ peak of ^{197}Hg at 77 keV. The original procedure was modified for use in the low-level analysis to increase sensitivity, eliminate interference, and diminish mercury loss and contamination risks. Sensitivity was increased by using a larger volume of sample (3 or 10 ml ampoules as against 0.3 ml used normally) and increasing the neutron irradiation dose by a factor of 5.

Interference from small amounts of gold, which may follow mercury through the chemical separation, is due to ^{198}Au peaks in the area of 77 keV. The interference was corrected for by measuring the ^{198}Au peak at 412 keV, multiplying by an experimentally determined factor and subtracting from the

total measured area of the 77 keV peak. For 88% of the samples the ^{198}Au peaks were less than 50% of the total peak. For a single sample it was more than 80% of the total peak. Loss of sample mercury which may occur during crushing of the quartz ampoules, was avoided by cooling the rinsed ampoules in liquid nitrogen before breaking. The risk of contamination of the samples in the laboratory was minimised by using new polyethylene beakers for each electrolysis, and by carrying out all operations on low level samples in an isolated clean laboratory with separate glassware.

The mercury content in the 28 glacial samples from 1727 to 1971 varies between 2 ng per kg and 19 ng per kg, with the lower limit being the sensitivity limit of the analytical method (Table 1).

The reliability of the analytical method was ascertained by analyses of standards and intercalibrations with other laboratories.

Low level standards are not available, but a NBS standard, NBS-1642, with a certified mercury content of $1,180 \pm 50$ ng per kg (the lowest content of mercury in standard material) diluted 10 and 100 times by deionised water with a mercury content of 10 ± 4 ng per kg was used (Table 2). The standard is not recommended for dilution, mainly because of the risk of losing mercury by the change of pH and stabiliser concentration and the possibility of introducing mercury into the dilution agent. The results are, therefore, only a demonstration of the ability of the method to reproduce figures in the right order of magnitude at the levels dealt with in glacial ice. For that purpose the analytical results are found to correspond satisfactorily to the calculated values.

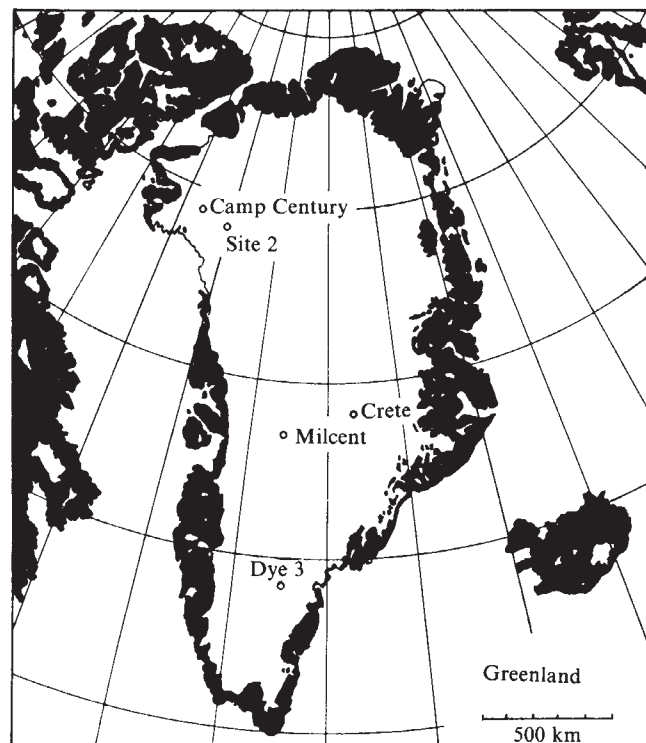
In an intercalibration programme on mercury in seawater undertaken by the International Council for the Exploration of the Sea in 1976 (ref. 9), 14 international laboratories participated, and five were able to determine the correct order of magnitude of mercury content in unpolluted unfiltered North Atlantic ocean water with a mercury content at about 5 ng per kg. The DIC found 13 ± 4 ng per kg and 3 ± 2 ng per kg in samples directly sucked in evacuated ampoules at the site and 31 ± 3 ng per kg and 6 ± 3 ng per kg in a sample taken by normal water sampler and sent to DIC's laboratory in bottles. The high value was obviously caused by contamination, and reanalyses of two samples gave 11 ± 3 ng per kg and 7 ± 3 ng per kg. The finding stresses the need for extreme care in the sampling and analytical processes when handling samples with ultra low mercury content. In a spiked sample at 131 ng per kg the DIC found 131 ± 7 ng per kg and 121 ± 6 ng per kg; 11 of the participant laboratories were able to determine this level.

Blank values for empty irradiated quartz ampoules have shown that the detection limit of the method is 0–3 ng per kg. By changing the cleaning procedure of the ampoules by heating the ampoules in an oven at 500°C instead of cleaning with acid, the detection limit can be lowered to less than 1 ng per kg.

The test analyses prove that the analytical results on the ice cores are of the right order of magnitude, and not caused by loss in the process. The finding is about one order of magnitude lower than that found by Weiss *et al.*¹ and Carr and Wilkniss³ at the Camp Century area, and about two orders of magnitude lower than found by Herron *et al.*⁵ at Milcent. This could indicate variations of orders of magnitude from one to another part of Greenland. Our analytical results of Dye 3 and Crete cores are not significantly different and do not support this statement, even though the two sites are separated by a distance of more than 700 km, with different meteorological conditions at the two sites. Our findings in the deposits of 1902 and 1905 from Dye 3 of 6 ng per kg and 7 ng per kg, respectively, might question the mercury concentration at 78 ng per kg found in 1900 deposits of Dye 3 by Weiss *et al.*⁴.

Volcanism is known to be a contributing factor to the mercury in the environment. In 16 samples of surface seawater adjacent to the lava front of the volcanic eruption at Heimaey, Iceland¹⁰ concentrations varying between 37 and 478 ng per l seawater were found. A few hundred metres off the front, the

Fig. 1 Map of Greenland with the ice core drilling sites used for mercury determinations. Our samples were collected at Crete and Dye 3. Weiss *et al.*¹ and Carr and Wilkniss³ analysed samples from the Camp Century region, Weiss *et al.*⁴ from Dye 3 and Herron *et al.*⁵ from Milcent. □, Glaciers; ■, Land.



concentration in six samples varied between 5 and 10 ng per l seawater, the same as we found in unpolluted seawater. It is surprising that Herron *et al.*⁵ found more than twice the concentration of the mercury-polluted seawater adjacent to the lava front in the Milcent samples.

Samples of the twentieth century were chosen here to obtain a time series of samples originating from one single site for investigation of whether or not any recent increase in the mercury content could be seen². The eighteenth century was chosen to demonstrate whether mercury introduced to the atmosphere by degassing of fluid magma from the volcanic eruption of Laki, Iceland, in 1783 had caused increased mercury content¹¹ in the Greenland Ice Cap. For these purposes the Crete ice core was superior because of the well-dated deposits, undisturbed up to recent years. The analyses of the Dye 3 samples were carried out as pilot studies on the sampling method.

In samples from the twentieth century a mean mercury content of 11 ng per kg was found based on seven samples from the period 1938–71. In three samples of the period 1926–37 the content decreased to 5 ng per kg, but a peak at 15 ng per kg was found in the 1913 deposit. The mercury content in deposits of the eighteenth century was of the same order of magnitude as that of the twentieth century, with a low level at about 5 ng per kg in deposits from 1775–86, and a peak at 14 ng per kg in deposits from 1772–74.

The eruption of Laki seems to be revealed only in the deposits of 1783 by a significant increase in the electrical conductivity of the ice¹². The event has not caused a simultaneous nor subsequent increase in mercury deposition in the

Table 2 Mercury analysis of a certified NBS standard, NBS-1642

Certified and calculated Hg values (ng per kg)	Analysed values (ng per kg)	No. of analyses
1,180 ± 50	1,077 ± 39	7
127 ± 16	117 ± 11	6
22 ± 6	16 ± 2	6

Undiluted and diluted 10 and 100 times by deionised water with a mean mercury concentration of 10 ± 4 ng per kg determined twice.

ice. Following the volcanic chronologies of Lamb¹³ and Cronin¹⁴, the measured peak values of mercury could not be correlated with volcanic events. The mercury content found in the arctic ice has been used as essential information on the atmospheric mercury burden¹. Thus, our findings question the validity of previous calculations of the global mercury turnover.

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- Weiss, H. V., Koide, M. & Goldberg, E. D. *Science* **174**, 692 (1971).
- Dickson, E. M. *Science* **177**, 536 (1972).
- Carr, R. A., & Wilkiss, P. E. *Science* **181**, 843 (1973).
- Weiss, H., Bertine, K., Koide, M. & Goldberg, E. D. *Geochim. cosmochim. Acta* **39**, 1 (1975).
- Herron, M. M., Langway, C. C. Jr, Weiss, H. V., Husley, P., Kerr, R. & Cragin, J. H. *Int. Symp. Isotopes & Impurities in Snow & Ice*, Grenoble (1975).
- Johnsen, S. J., Dansgaard, W., Clausen, H. B. & Langway, C. C. Jr. *Nature* **235**, 429; **236**, 249 (1972).
- Hammer, C. U. *Int. Symp. Isotopes & Impurities in Snow & Ice*, Grenoble (1975).
- Sjöstrand, B. *Analyt. Chem.* **36**, 814 (1964).
- Olafsson, J. *Mar. Chem.* **6**, 87–95 (1978).
- Olafsson, J. *Nature* **255**, 138 (1975).
- Siegel, B. Z., Siegel, S. M. & Thorarinnsson, F. *Nature* **241**, 526 (1973).
- Hammer, C. U. *Nature* **270**, 482 (1977).
- Lamb, H. H. *Phil. Trans. R. Soc. Lond. A* **266**, 425 (1970).
- Cronin, J. F. *Science* **172**, 847 (1971).

Table 1 Mercury concentrations in the glacial samples of Crete (C) and Dye 3 (D) stations

Site	Time of deposition	Mercury (ng per kg)
C	1971	9/13*
C	1970	7/10
C	1969	11/12
C	1964	16/10
C	1960	12/12
C	1957	11/12
C	1938	12/10
C	1937	6/8
C	1936	2/3
C	1926	6/7
C	1913	14/15
D	1905	4/8†
		4/7†
D	1902	8/8†
		6/5†
C	1786	2/5
C	1785	3
C	1783	4/3
C	1782	5/2
C	1781	5/4
C	1780	4/5
C	1779	(12)/6
C	1778	(6)/5
C	1777	(7)/3
C	1776	5/4
C	1775	4/5
C	1774	13/8, 8‡/14‡
C	1773	17/18
C	1772	11/11, 7‡/19‡
D	1727	8/3/3

* Solidus indicates individual results from two analyses on each sample. 3 ml of Dye 3 samples were used for analysis with a standard deviation, s.d. < 2 ng per kg. 10 ml of Crete samples were used with a s.d. < 1 ng per kg. Figures in brackets have a s.d. of about 6 ng per kg. All s.d. based on counting statistics of individual analyses.

† Half-year deposits.

‡ Samples sucked directly into evacuated ampoules.

Sexual imprinting and optimal outbreeding

WHEN Konrad Lorenz showed that early experiences can have a dramatic influence on the mating preferences of birds, he proposed that 'sexual imprinting', as the process came to be known in English, enables adults to recognise their own species¹. Subsequent experimental work^{2,3} has, however, suggested that, although early experiences can indeed have long-lasting effects, a bird may also show a preference for members of its own species in the absence of experience with any of them except itself. The implication is that a bird may have a predisposing bias for its own species and sexual imprinting merely refines this bias in natural conditions. If birds are able to identify and mate with their own species without prior experience (and seeing or hearing themselves is irrelevant)

what is the biological function of sexual imprinting? One possible answer is that sexual imprinting is required for recognition of close kin so that, by selecting mates that are slightly different, the animal is able to strike an optimal balance between inbreeding and outbreeding⁴. The prediction is that the strongest mating preference of a bird should be for something a little different (but not too different) from the object with which it had been imprinted. I report here that male Japanese quail (*Coturnix coturnix japonica*) mate with slightly unfamiliar females in preference to females to which they were exposed in early life, and that both types of female are preferred to those with a grossly unfamiliar type of plumage.

The quail were raised from hatching in four randomly assorted groups, each consisting of 10 chicks. At 35 d after hatching 20 males were isolated and when they were sexually mature, were given two types of test between 59 and 83 d after hatching. First, they were given an opportunity to approach females placed in the corners of a triangular cage behind perspex windows. Second, the males were placed in an open cage with two females and given 10 opportunities to copulate.

The males' mean scores are given in Table 1. One approach test was between a familiar female and an unfamiliar female of the same brown plumage as the pseudo-sister. The same pair of females was used for several tests, and males which were familiar with one female were tested in alternation with males familiar with the other. This procedure was used to counter-balance any differences in the females' attractiveness which were not connected with their relative familiarity. The ratio of time spent within 15 cm of the familiar female to the total time spent within 15 cm of both females in a 10-min test was significantly below the chance level. As the males apparently preferred unfamiliar females, they were given a choice between an unfamiliar female of the same brown plumage as their pseudo-sisters and an unfamiliar female with white plumage. The approach ratio was significantly above the chance level. In other words, they strongly preferred the female with brown plumage and there were clear limits to their appetite for novelty.

In one copulation test each male was placed in a cage with two brown females, one of which had been in the same rearing group as the male. Once the male grasped the neck feathers of a female and mounted, his choice was recorded and the test was ended by turning out the lights for 15 s. He was tested 10 times. Once again males familiar with one female were alternated with males familiar with the other. The preference for the familiar female was significantly below the chance level. In the other copulation test the males were given a choice between a familiar brown female and an unfamiliar white female. The preference for the familiar was significantly above the chance level.

The tests showed that the male quail strongly preferred brown females to white ones. Although this might indicate that

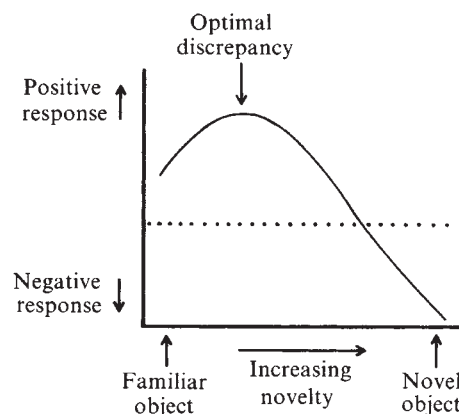


Fig. 1 The postulated relationship between the sexual response to an individual and its degree of novelty relative to familiar objects which would be kin in natural conditions.

white plumage is intrinsically less attractive, other experiments have shown that male Japanese quail exposed to white females in early life will prefer to mate with them rather than with brown females^{5,6}. Even so, the most striking finding of the present study was that the males' strongest mating preferences were for unfamiliar brown females. This suggests that in natural conditions the mating preferences of quail could be influenced by their experience with their siblings and that an important function of imprinting may be to enable birds to strike an optimal balance between inbreeding and outbreeding. I am now investigating the extent to which the females play a part in the process of pairing; results from other species suggest that females' preferences can also be influenced by their early experience⁷.

My general suggestion is that many animals may learn the visual, auditory or even olfactory characteristics of their immediate kin and opt to mate with an individual that is slightly different from those that are familiar from early life (see Fig. 1). The extent of the discrepancy would presumably depend on an inherited rule which in turn would have arisen from the opposing evolutionary pressures to be well adapted on the one hand and to be highly adaptable on the other⁸. If sexual imprinting provides the means by which an optimal balance between inbreeding and outbreeding is achieved, it follows that it should involve the learning of each siblings' appearance at a time when it has acquired enough of its adult plumage to be recognised subsequently. The available evidence about the stage of development when early exposure influences mating preferences suggests that this may happen⁹. Furthermore, the results reported here indicate that male quail, at least, are able to recognise individually each of their female siblings.

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Table 1 Choices between two females by male Japanese quail

Test	Choice		Score
	a	b	
Approach	Familiar brown	Novel brown	0.25 ± 0.06*
	Novel brown	Novel white	0.84 ± 0.04*
Copulation	Familiar brown	Novel brown	3.8 ± 0.4†
	Familiar brown	Novel white	9.4 ± 0.2*

Means and standard errors are given for 20 males. The measure for the approach test is the time near *a* divided by the total time near *a* and *b*; the chance level is 0.5. The measure for the copulation test is the number of copulations with *a* out of 10 choices between *a* and *b*; the chance level is 5. Each individual's score was compared with the chance level and values of matched pairs *t* calculated.

**P* < 0.001.

†*P* < 0.02.

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- Lorenz, K. J. *Orn. Lpz.* **83**, 137–213; 289–413 (1935).
- Immelmann, K. Z. *Tierpsychol.* **26**, 677–691 (1969).
- Schutz, F. Z. *Tierpsychol.* **22**, 50–103 (1965).
- Bateson, P. P. G. in *Biological Determinants of Sexual Behaviour* (ed. Hutchison, J. B.) 29–53 (Wiley, London, 1978).
- Gallagher, J. *Behaviour* **57**, 91–114 (1976).
- Gallagher, J. J. *comp. physiol. Psychol.* **91**, 72–78 (1977).
- Sonnemann, P. & Sjölander, S. Z. *Tierpsychol.* **45**, 337–348 (1977).
- Mather, K. *Genetical Structure of Populations* 1–197 (Chapman and Hall, London, 1973).
- Bateson, P. P. G. *Anim. Behav.* (in the press).

Relationship between heterozygosity for enzyme loci and variation of morphological characters in natural populations

THERE is little consensus among evolutionary biologists on the roles of morphological and protein variation within populations, and little consideration is given to their covariation. Morphological variation is known to be influenced by both the genetics of individuals and the environments in which they develop. Crosses between domesticated strains of plants and animals suggest that highly heterozygous individuals have enhanced developmental homeostasis¹, but the implications of this phenomenon for natural populations have not been extensively explored. Reported here is an examination of the relationship between genetic heterozygosity of proteins and morphological variation in natural populations of the killifish, *Fundulus heteroclitus*. Results indicate that individuals heterozygous for an enzyme locus are likely to be less morphologically variable than individuals homozygous for that locus.

In an effort to understand more about the role of genetic variation in natural populations, population geneticists have measured levels of polymorphism and heterozygosity for proteins, and have tried to relate these measurements to the position of the population in the species range², amplitude of environmental variation³, predictability of trophic resources⁴, and niche breadth⁵. Phylogenetic trees derived from allozyme data and morphometric data gathered from six species of *Menidia* are nearly identical, suggesting that similar evolutionary forces act on protein polymorphisms and morphological variation⁶. Yet few studies have attempted to relate the level of polymorphism or heterozygosity to the amount of morphological variation. The number of loci polymorphic in a

population is related to the variance of meristic characters of the side-blotch lizard, and the number of dermatoglyphic patterns in macaques^{7,8}. In each case, greater phenotypic diversity is associated with a greater number of genotypes. In addition, a diversity of observations suggest that higher levels of heterozygosity confer enhanced canalisation or developmental homeostasis, resulting in lower levels of phenotypic variance¹. Some of the clearest examples of this relationship come from laboratory experiments in which the level of heterozygosity is manipulated, and one or more measures of phenotypic variation are monitored⁹⁻¹¹. These two relationships are not contradictory, but represent, respectively, inter-population and intrapopulation phenomena. That is, within any population, the least phenotypic variation will exist in the most heterozygous individuals, but populations with a greater diversity of genotypes, all else being equal, will have greater phenotypic variance.

The intrapopulation relationship between heterozygosity and phenotypic variance has until now been documented primarily with manipulated stocks. However, the capability now exists to test this hypothesis in natural populations. This paper reports the results of a study that contrasts heterozygotes and homozygotes for their levels of phenotypic variance.

Data were taken from a study of geographical variation of enzyme polymorphisms and morphometric variation of the killifish, *Fundulus heteroclitus*, in Long Island Sound, New York¹². From a data set of twelve polymorphic proteins, five loci with the highest proportions of heterozygotes (serum esterase, SERE; liver esterase, EST-2; lactate dehydrogenase, LDH; glucose-6-phosphate dehydrogenase, G6PDH; phosphoglucosyltransferase, PGM-1) were chosen. Individuals subjected to genetic analysis were also characterised for their number of scales along the lateral line, below the lateral line, and around the caudal peduncle, and the number of fin rays in their dorsal, caudal, anal and pectoral fins. Population samples were available for three localities, and because of the possibility of sexual dimorphism and genetic differences between the sexes, males and females were analysed separately. For each locus, the

Table 1 Estimates of multivariate variance for heterozygous and homozygous classes of *Fundulus heteroclitus*

Locality and sex	Variable	SERE		EST-2		Loci LDH		G6PDH		PGM-1	
		Het	Hom	Het	Hom	Het	Hom	Het	Hom	Het	Hom
Flax Pond ♂♂	CV	5.62	5.67	5.84	5.60	5.53	5.82	5.75	5.55	5.71	5.73
	Σ	0.019	0.042	0.0007	0.045	0.032	0.055	0.027	0.044	0.033	0.056
	n	28	54	12	50	31	51	29	49	35	45
Flax Pond ♀♀	CV	5.50	5.63	5.49	5.72	5.43	5.65	5.83	5.35	5.55	5.60
	Σ	0.043	0.057	0.017	0.062	0.024	0.064	0.063	0.032	0.048	0.051
	n	54	121	35	105	54	123	76	98	81	89
Northport ♂♂	CV	6.13	5.51	5.37	5.90	5.62	5.82	5.75	5.80	5.50	5.87
	Σ	0.074	0.059	0.025	0.112	0.036	0.098	0.047	0.080	0.039	0.089
	n	32	77	32	79	42	69	33	73	43	67
Northport ♀♀	CV	5.44	5.49	5.66	5.52	5.44	5.56	5.56	5.59	5.42	5.67
	Σ	0.022	0.049	0.031	0.043	0.033	0.042	0.024	0.055	0.035	0.040
	n	48	143	48	143	71	120	53	124	65	122
Centerport ♂♂	CV	4.82	5.64	5.42	5.28	5.11	5.37	5.15	5.42	5.25	5.48
	Σ	0.001	0.029	0.015	0.005	0.0002	0.012	0.011	0.005	0.004	0.011
	n	18	27	28	17	14	31	26	19	20	25
Centerport ♀♀	CV	5.71	5.38	5.39	5.58	5.75	5.31	5.19	5.59	5.46	5.48
	Σ	0.007	0.013	0.011	0.013	0.006	0.013	0.006	0.016	0.010	0.015
	n	14	40	35	19	20	34	30	24	25	29

CV, Average coefficient of variation; |Σ|, determinant of the variance-covariance matrix; n, sample size, het and hom represent heterozygous and homozygous groups. Information may be taken from the table after the following example. Thirty-one of the males from Flax Pond were heterozygous for LDH, and 51 were homozygous. Phenotypic variation of the heterozygotes was 5.53 when estimated using the mean coefficient of variation, and 0.032 when estimated using the determinant of the variance-covariance matrix. Corresponding values for homozygotes are 5.82 and 0.055. Thus, both measures indicate that individuals heterozygous for this locus tend to be less variable than homozygous individuals.

population sample was divided into groups, those individuals heterozygous for that locus, and those homozygous. Phenotypic variance for the heterozygous and homozygous groups was estimated using all seven meristic variables with the average coefficient of variation or population parameter of Soulé¹³ and the determinant of the variance-covariance matrix¹⁴.

The null hypothesis tested here is that individuals heterozygous for an enzyme locus have the same level of morphological variation as individuals homozygous for that locus. There are at present no standard errors or tests of significance for either the average coefficient of variation or the determinant of the variance-covariance matrix, so that differences cannot be tested directly. Yet rejection or support of the null hypothesis may be judged by the sign test¹⁵, evaluating the number of comparisons in which heterozygotes have lower phenotypic variance than homozygotes. When heterozygotes are compared with homozygotes using the mean coefficient of variation (Table 1), heterozygotes have lower phenotypic variation than homozygotes in 22 of 30 tests ($P < 0.05$). The determinant of the variance-covariance matrix gives more striking results, heterozygotes being less variable than homozygotes in 26 of 30 tests ($P < 0.001$).

Several of the loci surveyed in this analysis have been postulated to have an impact in the determination of fitness¹², but the evidence for lactate dehydrogenase (LDH) is most convincing. Gene frequencies differ between populations in artificially heated and control environments, and this observation is consistent with differences in gene frequencies between populations from natural environments that differ in temperature. Furthermore, kinetic differences between LDH genotypes are consistent with the observed geographical variation in allelic frequencies¹⁶. Yet in this study, as in most studies, it is not possible to discern whether the loci being analysed produce the differences observed, or whether they function in this analysis as markers of segments of chromosomes.

When soluble proteins were first used by population geneticists to assess levels of genetic variation, these markers were presented as a random sample of the genome¹⁷. Since then, several observations suggest, for a variety of reasons¹⁸⁻²², that groups of enzymes differ in their levels of genetic variation. If groups of proteins differ in their levels of genetic variation, it is not likely that electrophoretically detectable genetic variation will give an accurate assessment of the total genetic variation of a population. Given this heterogeneity and doubt about just what is being measured, and further doubt concerning the adaptive value of protein variation²³, the results reported here are surprising. On the basis of genetic variation of a single locus, a population can be subdivided into two groups that differ in their levels of morphometric variation (Table 1). This dissection of a population is replicable in three localities, for five loci tested.

This first observation is consonant with observations compiled by Lerner¹, who extensively documented the relationship between higher heterozygosity and enhanced developmental homeostasis, but it raises several other questions. Is it the single protein locus that influences developmental homeostasis, is it loci linked to this locus, or is it heterozygosity correlated with this locus? Does normalising selection acting on morphological variation impinge on protein polymorphisms that influence developmental homeostasis? These data suggest that heterozygosity influences developmental homeostasis, but the source of this effect, and the impact of this effect on evolution is yet to be resolved.

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1. Lerner, I. M. *Genetic Homeostasis* (Oliver and Boyd, Edinburgh, 1954).
2. Soule, M. A. *Rev. Sys. Ecol.* **4**, 165-187 (1973).
3. Somero, G. N. & Soule, M. *Nature* **249**, 670-672 (1974).
4. Ayala, F. J., Valentine, J. W., Hedgecock, D. & Barr, L. G. *Evolution* **29**, 203-212 (1975).
5. Sabath, M. D. *Am. Nat.* **108**, 533-540 (1974).
6. Mickevich, M. F. & Johnson, M. S. *Syst. Zool.* **25**, 260-270 (1976).
7. Soule, M. *Taxon* **20**, 37-50 (1971).
8. Morris, L. N. & Kerr, B. A. *J. hum. Evol.* **3**, 223-235 (1974).
9. Mather, K. *Biometrical Genetics* (Methuen, London, 1949).
10. Robertson, F. W. & Reeve, E. C. R. *Nature* **170**, 286-287 (1952).
11. Dobzhansky, Th. & Wallace, B. *Proc. natn. Acad. Sci. U.S.A.* **39**, 162-171 (1953).
12. Mitton, J. B. & Koehn, R. K. *Genetics* **79**, 97-111 (1975); *Biol. Bull.* **151**, 548-559 (1976).
13. Soule, M. *Am. Nat.* **106**, 429-446 (1972).
14. Sokal, R. R. *Biol. Rev.* **40**, 337-391 (1965).
15. Sokal, R. R. & Rohlf, F. J. *Biometry* (Freeman, San Francisco, 1969).
16. Powers, D. A. & Powers, D. in *Isozymes 4: Genetics and Evolution* (ed. Markert, C. L.) 63-84 (Academic, New York, 1975).
17. Lewontin, R. C. & Hubby, J. L. *Genetics* **54**, 595-609 (1966).
18. Kojima, K., Gillespie, J. H. & Tobari, Y. N. *Biochem. Genet.* **4**, 627-637 (1970).
19. Johnson, G. B. *Science* **184**, 28-37 (1974).
20. Zouros, E. *Nature* **262**, 227-229 (1976).
21. Ward, R. D. *Biochem. Genet.* **15**, 123-135 (1977).
22. Koehn, R. K. & Eanes, W. F. *J. theor. Pop. Biol.* **11**, 330-341 (1977).
23. Kimura, M. & Ohta, T. *Theoretical Aspects of Population Genetics* (Princeton University Press, 1971).

Immunological memory is regulated in the enhanced rat renal allograft recipient

SUCCESSFUL transplantation of renal allografts without compromising the immune system of the recipient is a goal of clinical transplantation. The enhancement of kidney allografts in the inbred rat provides an experimental system satisfying this goal. Pretreatment of recipient animals with antigen and antibody, alone or in combination, has produced the indefinite survival of renal allografts¹⁻⁴, but the mechanisms underlying this specific elimination of responsiveness to the donor graft have not been completely elucidated. Three alternative mechanisms have been proposed to explain the maintenance of the enhanced state: (1) deletion or alteration of donor antigens on the graft; (2) deletion of the relevant antigen-reactive cells; (3) regulation of the host response to the graft. We discuss these suggestions here, and report our own work on enhancement, giving evidence for the role of immunological memory regulation.

Deletion of graft antigens does not account for the maintenance of the enhanced kidney. Antigen in a form and amount sufficient to lead to rejection persists in the donor graft. Stuart *et al.* have shown that an enhanced Lewis × Brown Norway (LBN) F₁ hybrid kidney removed from an enhanced Lewis rat and retransplanted into a normal untreated Lewis recipient is rejected in only a slightly delayed fashion⁵. Furthermore, the accessibility of donor graft antigens in the enhanced animal has been demonstrated by *in vivo* antibody absorption studies⁶.

Alteration of donor graft antigens most certainly occurs in the long-term enhanced kidney through the loss of 'passenger leukocytes'. Passenger leukocytes provide a potent antigenic stimulus in the donor allograft and may be a sufficient antigenic stimulus alone to cause the rejection of a renal allograft⁷. Some kinds of leukocytes bear Ia antigens which may be necessary for, or at least augment the activation of amplifier T cells⁸. These amplifier T cells can heighten the response of killer T cells which react mainly with antigens defined by other regions of the major histocompatibility complex⁸. Batchelor *et al.* have proposed that kidney allografts in enhanced recipients are depleted of passenger leukocytes and thus are deficient in Ia antigens needed for the activation of amplifier T cells⁹. These authors suggest that enhanced kidneys are unable to stimulate an effective host response due to the deficit of Ia antigens and the suppressive effects of continued exposure to other MHC antigens. This explanation is supported by the observations that have been made in the thyroid allograft model in the mouse. The previous depletion of passenger cells by short-term *in vitro* culture results in successful long-term transplantation of thyroid allografts in mice, and injection of leukocytes bearing graft-type alloantigens results in rejection of the thyroid

graft¹⁰. However, two findings indicate that this mechanism cannot account for renal allograft survival in enhanced recipient rats. As noted above, enhanced kidney allografts are rejected when retransplanted into untreated rats syngeneic with the original recipient⁵. Furthermore, fresh kidney allografts from the same strain as the original enhanced kidney and bearing a full complement of passenger leukocytes are accepted indefinitely by long-term enhanced rats without any additional treatment⁵. Thus, the maintenance of the enhanced state in the rat renal allograft model cannot be explained simply by deletion or alteration of donor graft antigens present either in the kidney or on passenger leukocytes.

Deletion of primary antigen-reactive cells in the host seems not to account for the maintenance of the enhanced kidney. Normal proliferative responses have been obtained with lymphoid cells from enhanced rats in local graft-versus-host assays as well as in mixed leukocyte cultures (MLC) (refs 11–14). Cytolytic responses towards the donor antigens in MLC have been observed with spleen cells from long-term enhanced rats, and the magnitude of the response is comparable to that obtained with spleen cells from normal rats¹⁴. However, the reactivity of these cell populations has been studied either in normal rats or in tissue culture. A limited number of experiments (3) has been performed to determine whether these cells can develop such reactivity in the enhanced animal, and it has not been definitively shown that such reactivities reflect the functional cell population(s) important in the rejection of an allograft. Nevertheless, the normal reactivity *in vitro* of lymphoid cells from enhanced rats towards the graft antigens indicates that antigen-reactive cells are not deleted in the recipient bearing an enhanced kidney allograft.

As neither deletion or alteration of graft antigens nor deletion of reactive cells account for the enhanced state, regulation of the host response to the donor graft antigens is the only tenable hypothesis. To characterise the regulatory mechanisms involved in allograft enhancement, we examined the reactivity of enhanced rats to a fresh challenge of donor antigen.

Long-term enhanced renal allograft survivors were prepared by pretreating Lewis rats with 5×10^7 LBN spleen cells and, 24 h later, with 1 ml of Lewis anti-BN serum. After 10 d, these rats were bilaterally nephrectomised and an LBN kidney was transplanted. Most rats receiving this treatment survive indefinitely without signs of rejection¹². All long-term enhanced rats used in this study had survived 35 d or longer without elevation of their blood urea nitrogen (BUN).

We evaluated the reactivity of these animals to donor antigens using a local popliteal node host-versus-graft assay¹⁵. Normal Lewis or long-term enhanced Lewis rats received either 10^7 Lewis, LBN or Lewis $\times$ ACI (LACI) F₁ spleen cells in the hind footpads. Local popliteal nodes were removed after 5 d and weighed to assess node enlargement. As Table 1 indicates, the response of long-term enhanced rats to donor as well as third party alloantigens present on spleen cells was

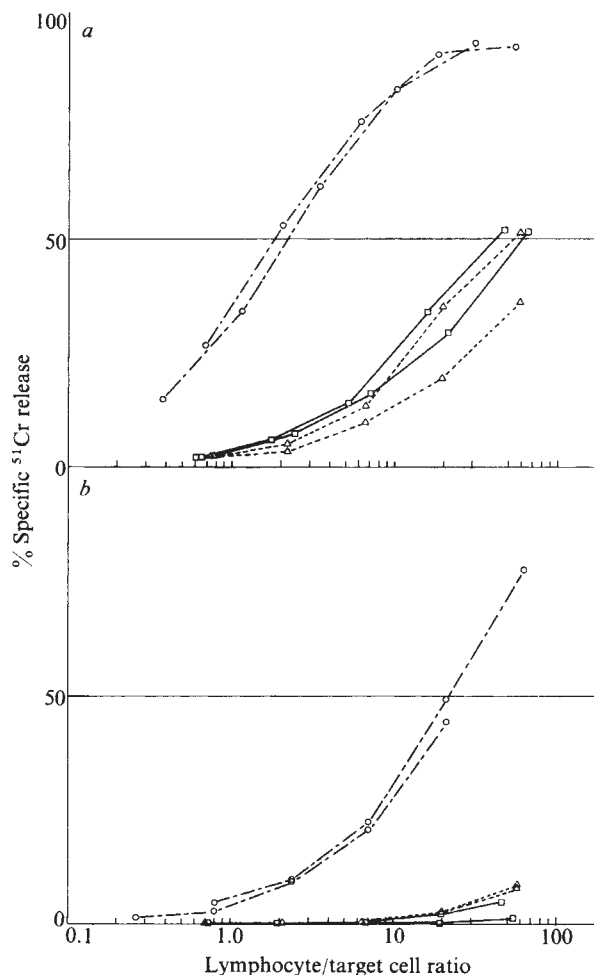


Fig. 1 Generation of cytolytic activity in cultures prepared with spleen cells from normal Lewis rats (□), or Lewis rats (○) and long-term enhanced renal allograft recipients (Δ) challenged with LBN spleen cells subcutaneously 3 weeks previously. MLC were prepared with carbonyl iron-treated spleen cells from two animals of the indicated group and either X-irradiated BN cells (a) or subcellular BN antigen (b). Cytolytic activity generated was assessed after 7 d in a 6-h ⁵¹Cr release assay using cloned BN lymphoma targets. Subcellular antigen preparations were used to distinguish further the secondary CTL precursors from the primary CTL precursors¹⁸.

nearly equivalent to that of normal rats, as shown by the increase in popliteal node weights. Thus, the enhanced renal allograft recipient can recognise and respond to donor alloantigens on leukocytes. In other experiments, long-term rats were injected with donor leukocytes subcutaneously, and BUN was measured frequently over a period of 30 d. No increase in BUN was observed. Thus, despite the ability of rats bearing enhanced renal allografts to respond to donor antigens on leukocytes, the activation of antigen-reactive cells in these rats did not lead to the rejection of the kidney allograft.

We examined whether memory cells for cell-mediated immune responses were generated in enhanced renal allograft recipients which had been challenged with antigen. MLC were prepared with spleen cells from normal Lewis rats, or normal Lewis rats and long-term enhanced Lewis rats which had been injected subcutaneously three weeks previously with 10^7 LBN spleen cells. Secondary cytolytic responses were obtained with spleen cells from normal Lewis rats which had been challenged with LBN spleen cells. However, the level of cytolytic activity generated in cultures prepared with cells from long-term enhanced rats which had been challenged with LBN antigen were comparable with those generated in cultures of normal spleen cells (Fig. 1). Secondary cytolytic responses were obtained with cells from long-term enhanced rats when the

Table 1 Local host-versus-graft response of normal Lewis and long-term renal allograft recipients*

Animal injected	Popliteal lymph node weight (mg) Spleen cells injected		
	Lewis	LBN	LACI
Normal Lewis			
Group 1	6.4 ± 0.4	35.2 ± 5.1	
Group 2		33.4 ± 0.6	42.3 ± 0.9
Lewis with long-term enhanced LBN kidney			
Group 1	7.2 ± 1.4	29.6 ± 2.0	
Group 2		25.9 ± 1.9	26.4 ± 3.0

* Normal Lewis or Lewis rats bearing enhanced LBN renal allografts received in the footpad 10^7 spleen cells from the indicated donor. After 5 d the ipsilateral popliteal nodes were weighed. Values are means ± s.e.m.

response to third-party antigen (LACI) was examined (data not shown). Thus, an inability to develop or maintain memory cells for cell-mediated responses such as generation of cytolytic T lymphocytes (CTL) is an apparent defect in the long-term enhanced rat. These results are particularly interesting in relation to the results obtained previously with skin grafts of long-term enhanced rats. Although LBN skin grafts were rejected in only a slightly prolonged fashion⁵, rejection of a second LBN skin graft was further prolonged in enhanced rats in contrast to the accelerated rejection of second set skin grafts in normal Lewis rats (F.P.S., unpublished data).

Although donor antigens on leukocytes are recognised and antigen reactive cells are activated in the enhanced rat, the subsequent development or maintenance of memory CTL precursors seems not to occur. These results demonstrate that the host immune response to donor antigens is regulated in long-term enhanced rat renal allograft recipients. This defect in development of immunologic memory may be important for the maintenance of the enhanced state. Memory CTL may represent a highly differentiated population of cells important in the rejection of renal allografts because of their heightened cytolytic activity. Failure to develop memory may be sufficient to account for the survival of renal allografts. Indeed, rejection of most tissue allografts is accompanied by the appearance of memory cells.

The mechanisms accounting for the failure to develop immunological memory are not known. Although anti-idiotypic antibody has been detected in the serum of antigen and antibody pretreated Lewis rats at the optimal time for transplantation,¹² a direct role for such antibody in enhancement or in the suppression of memory cells has not been shown. Occasionally, suppressive activity was observed when spleen cells from long-term enhanced rats were adoptively transferred to new renal allograft recipients¹⁶. Batchelor *et al.* were unable to detect suppressor cell activity in rats bearing enhanced renal allografts⁹. However, their interpretations seem somewhat limited, as skin graft survival was used to measure suppressor cell activity from renal allograft recipients, and it is well known that skin grafts are not readily accepted by long-term enhanced renal allograft recipients⁵. The possible role of suppressor cells in prevention of immunological memory is now under investigation. An alternative mechanism for the failure to develop immunological memory could involve the opsonisation of antigen-reactive cells in long-term enhanced rats, as proposed by Hutchinson and Zola¹⁷. As normal primary responses are obtained with cells from long-term enhanced rats, such a mechanism would have to eliminate selectively only memory cells that might be generated. Whatever the mechanism, regulation of the host immune response seems to be an important and perhaps major process involved in rat renal allograft enhancement.

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1. Stuart, F. P., Saitoh, T. & Fitch, F. W. *Science* **160**, 1463-1465 (1968).
2. French, M. E. & Batchelor, J. R. *Transplant Rev.* **13**, 115-141 (1972).
3. Batchelor, J. R. & Welsh, K. I. *Br. med. Bull.* **32**, 113-117 (1976).

4. Carpenter, C. B., d'Apice, A. J. B. & Abbas, A. K. *Adv. Immun.* **22**, 1-65 (1976).
5. Stuart, F. P., Fitch, F. W. & Rowley, D. A. *Transplant Proc.* **2**, 483-488 (1970).
6. French, M. E. *Transplant Proc.* **5**, 62-1623 (1973).
7. Guttman, R. D., Lindquist, R. R. & Ockner, S. A. *Transplantation* **8**, 472-484 (1969).
8. Cantor, H. & Boyse, E. A. *J. exp. Med.* **141**, 1376-1389, 1390-1399 (1975).
9. Batchelor, J. R., Brent, L. & Kilshaw, P. J. *Nature* **270**, 522-524 (1977).
10. Lafferty, K. J., Bootes, A., Dart, G. & Talmadge, D. W. *Transplantation* **22**, 138-149 (1976).
11. Ockner, S. A., Guttman, R. D. & Lindquist, R. R. *Transplantation* **9**, 39-48 (1970).
12. Stuart, F. P., Scollard, D. M., McKearn, T. J. & Fitch, F. W. *Transplantation* **22**, 455-466 (1976).
13. Sørensen, S. F., Bildsøe, P. & Simonsen, M. *Transplant Proc.* **4**, 181-187 (1972).
14. Weiss, A., Stuart, F. P. & Fitch, F. W. *Transplantation* (in the press).
15. Dorsch, S. E. & Roser, B. *Aust. J. exp. Biol. med. Sci.* **52**, 253-264 (1974).
16. Stuart, F. P., McKearn, T. J. & Fitch, F. W. *Surgery* **80**, 130-136 (1976).
17. Hutchinson, I. V. & Zola, H. *Transplant Proc.* **9**, 961-963 (1977).
18. Engers, H. D., Thomas, K., Cerottini, J. C. & Brunner, K. T. *J. Immun.* **115**, 356-359 (1975).

Immune response (*I*r) genes expressed at macrophage-B lymphocyte interactions

THERE are two major types of genetic control of antibody responses. One is linked to immunoglobulin (Ig) allotype, and the relevant genes are Ig heavy-chain variable-region genes, which control receptor structures on B cells¹. The other major region controlling immune responses lies in the major histocompatibility complex (MHC), a genetic region discovered due to its predominant influence on graft reactions, but also containing immune response (*I*r) genes². The mode of action of the MHC-linked *I*r genes is not known. Many hypotheses have been proposed both for their cellular site of action, that is, on T cells, B cells or macrophages, and for their mechanism of action²⁻⁴. Recently, we discussed the possibility that as macrophages are required for T and B lymphocyte activities, the available data were compatible with *I*r genes expressed at the

Table 1 Induction of response to (T,G)-A--L by F₁ or R macrophages from F₁ (R × NR) B cells *in vitro*

Antigen	Stimulus HF	PE cell	Anti-(T,G)-A--L response IgM AFC per 15 × 10 ⁶ ± s.e.
—	—	—	57 ± 7
+	—	—	47 ± 13
+	+	—	30 ± 15
—	—	F ₁	37 ± 3
+	—	F ₁	40 ± 10
+	+	F ₁	217 ± 34*
—	—	B10	13 ± 13
+	—	B10	47 ± 12
+	+	B10	193 ± 18*
—	—	B10.A	30 ± 15
+	—	B10.A	50 ± 6
+	+	B10.A	57 ± 18

Response of (B10 × B10.A)F₁B cells from mouse spleen to (T,G)-A--L. T cells were removed by lysis with sheep anti-mouse T-cell serum¹¹ and complement, and then macrophages were removed by using carbonyl iron¹¹. 40% of cells were lysed with anti-T cell serum, and a 50% cell loss with the carbonyl iron. Helper factor (HF) to (T,G)-A--L obtained from CBA helper cells made as described earlier²³ were used at a concentration of 1/1,000, batch 237, the optimum found previously⁹. Response was measured in Marbrook flasks, with 15 × 10⁶ cells cultured in 1 ml HEPES-buffered medium containing 5% foetal calf serum, and 1 μg ml⁻¹ of antigen, at day three. Sheep red cells were coated with (T,G)-A--L using CrCl₃, as described by Taussig²⁴ in a Cunningham type plaque assay²⁵. The efficiency of the anti-T serum treatment was monitored by inhibition of the anti-SRBC response *in vitro*, without inhibiting the response to DNP acrylamide bends²⁵. Macrophages used were from normal peritoneal washings; 3 × 10⁵ cells were added for the restoration. Three experiments of this type have been carried out with analogous results, using (B10 × B10.Br)F₁ mice also.

* *P* < 0.01, numbers statistically significantly different from the appropriate background by *t*-test. Background is control + appropriate PE cells + antigen alone.

Table 2 Induction of the response to GAT by F_1 or R macrophages from F_1 (R $\times$ NR) B cells *in vitro*

Antigen	Stimulus HF	PE cell	Response IgM AFC per $3 \times 10^6 \pm$ s.e.
—	—	—	0
+	—	—	13 ± 9
+	+	—	3 ± 3
—	—	F_1	0
+	—	F_1	30 ± 0
+	+	F_1	$147 \pm 22^*$
—	—	B10.A	20 ± 12
+	—	B10.A	17 ± 12
+	+	B10.A	$177 \pm 13^*$
—	—	B10.G	60 ± 35
+	—	B10.G	33 ± 3
+	+	B.10.G	23 ± 19

The response of F_1 (B10.A $\times$ B10.G) spleen cells depleted of macrophages by carbonyl iron. The helper factor used was from the CBA strain (HF 325), used at 1/1,000. The efficiency of macrophage depletion is controlled by the lack of response to HF unless F_1 or B10.A macrophages are restored. The antigen, GAT, was used at $1 \mu\text{g ml}^{-1}$; macrophages derived from normal peritoneal washings were used at 10^5 per culture of 3×10^6 cells in a 'mini Marbrook' culture system.

* $P < 0.01$, see legend to Table 1.

macrophage-lymphocyte interactions, with either T cells or B cells⁵. Results implying the involvement of macrophage *Ir* genes in the macrophage-T lymphocyte interaction have been reported in several species and systems, and include antigen induced proliferation in guinea pigs and mice^{6,7}, and delayed-type hypersensitivity responses⁸. We report here the first evidence for macrophage *Ir* genes at the macrophage-B cell interaction.

Synthetic polypeptides such as (T,G)-A--L (co-polymer of L (tyrosine, glutamic acid) coupled to D,L-alanine side chains on a poly-L-lysine backbone) and GAT (random copolymer of 60% glutamic acid, 30% alanine and 10% tyrosine) have been used for many studies of *Ir* gene function^{3,4}. We have used (T,G)-A--L⁹ and GAT for preparing antigen-specific helper factors (HF) to localise at which arm of the antibody response genetic defects lie⁹, in helper factor induction, or in its capacity to stimulate 'helper factor expression', in a manner analogous to Munro and Taussig³. These workers suggested that the incapacity of some non-responder strains to respond to HF was

a B-cell defect due to a lack of the appropriate B-cell binding site for HF. However, we have found that HF_{TGAL}, like HF to proteins reported previously¹⁰, acts on macrophages first and not on B cells directly (ref. 9 and S.H. and M.F., in preparation).

Thus, we wondered which of the two cells involved in the response to HF, the B cell or the macrophage (or both), was the site of the *Ir* gene defect. By using combinations of responder B cells and responder (R) or non-responder (NR) macrophages, we investigated whether there are *Ir* genes expressed at the macrophage involved in the macrophage-HF-B cell interaction. The results indicate that for the two synthetic polypeptides used there are macrophage *Ir* genes involved in this interaction, but do not exclude the possibility that there may also be *Ir* genes expressed in B cells.

To avoid complications with allogeneic B cells and macrophages, F_1 (R $\times$ NR) for example, F_1 (B10 $\times$ B10.A) (phenotypically (T,G)-A--L-responder) mouse spleen cells were used as the B-cell source. These were depleted of their macrophages by carbonyl iron treatment¹¹, and in some experiments of T cells by anti-T serum and complement. The capacity of these cells to be stimulated by HF_{TGAL} and (T,G)-A--L in the presence of either F_1 , NR or R macrophages was tested. Only F_1 or R macrophages were stimulatory (Table 1). This is in close analogy to similar T cell F_1 (R $\times$ NR) interactions with either R or NR macrophage (experiments initially carried out by Rosenthal and Shevach in guinea pigs⁶), and as this applies to both antigens tested (Tables 1 and 2), suggests the interesting generalisation that *Ir* genes function at macrophage-lymphocyte interactions. Analogous experiments have been carried out in delayed hypersensitivity reactions, involving T cells and macrophages⁸.

To demonstrate that the cell responsible for reconstitution of the response was indeed a 'classical' macrophage (that is, a radioresistant, Ig-ve, adherent cell) peritoneal exudate (PE) cells were purified by irradiation and adherence to plastic or depleted of their Ig+ve (B) cells (Table 3). Irradiated-adherent and Ig-ve cells are capable of restoring the response of macrophage-depleted spleen cells.

It is unlikely that the inability of NR macrophages to stimulate B cells is due to suppressor cell induction, as there is no difference in results whether T cells are present (Table 2) or absent (Table 1).

In a previous report involving one of the antigens used in this study, Kapp *et al.*¹² reported that non-responder macrophages functioned normally in inducing primary responses to GAT *in*

Table 3 Irradiated-adherent or Ig-negative peritoneal exudate cells restore AFC responses of macrophage-depleted spleen cells

Antigen	Stimulus HF	PE cell	IgM AFC response per $3 \times 10^6 \pm$ s.e.		
			SRC _{bgd}	SRC _{GAT}	GAT specific
Expt 1					
+	+	—	33 ± 2	67 ± 33	33 ± 24
+	+	Untreated	47 ± 12	233 ± 52	$187 \pm 41^*$
+	+	Ig-ve	80 ± 3	327 ± 18	$247 \pm 15^*$
+	+	Irr.Adh	20 ± 10	233 ± 7	$213 \pm 9^*$
Expt 2					
+	+	—	3 ± 3	40 ± 6	37 ± 3
+	+	Untreated	50 ± 30	347 ± 47	$297 \pm 26^*$
+	+	Irr.Adh	33 ± 18	300 ± 12	$267 \pm 24^*$
Expt 3					
+	+	—	20 ± 0	27 ± 7	7 ± 7
+	+	Untreated	57 ± 12	267 ± 24	$210 \pm 26^*$
+	+	Ig-ve	43 ± 13	250 ± 26	$207 \pm 38^*$

Response of F_1 (B10.A $\times$ B10.G) spleen cells depleted of macrophages and reconstituted with syngeneic unstimulated PE cells. 3×10^6 spleen cells + $1 \mu\text{g}$ antigen + HF $\pm 3 \times 10^4$ PE cells were cultured for 4 d. The antibody forming cell (AFC) response is determined by measuring the number of IgM plaques per culture against untreated sheep erythrocytes (SRC_{bgd}) and against antigen-coated sheep erythrocytes (SRC_{GAT}). Irradiated-adherent (Irr.Adh.) PE cells were prepared by irradiating unstimulated PE cells with 1500 R and adhering them overnight to plastic dishes. Ig-negative (Ig-ve) PE cells were prepared using the rosetting procedure of Parish *et al.*²⁶; no Ig-positive cells were detected in the Ig-negative fraction, although the treatment removed approximately 10% of the original PE cell preparation.

* $P < 0.01$, see legend Table 1.

vitro. The discrepancy may be due to the different techniques used, or in the response measured (IgG or IgM) or the complicating aspect of the allogeneic effect, for in the experiments of Kapp *et al.*, F_1 lymphocytes were not used, and hence there may have been a reaction of T cells against the allogeneic macrophages releasing stimulatory factors.¹³ However, Yono *et al.*¹⁴ have recently found macrophage *Ir* genes with GAT in the T-cell proliferation assay.

The incapacity of NR macrophages to stimulate responses sheds some light on the mechanism of B cell-HF-macrophage interaction. Based on analogies to the structure of polymeric thymus-independent antigens, it was suggested that it may be sufficient for HF to induce a suitable aggregation and presentation of antigen on the macrophage surface^{10,15}. This is unlikely to be the sole function, as it is known that the non-responder macrophages do bind HF_{TGAL} in an immunogenic manner, provided that DNP (T,G)-A--L is used (S.H. and M.F., unpublished). Thus, it seems that macrophage participation is more active, and linked to the *Ir* gene function. It is tempting to speculate, based on the capacity of NR macrophages to bind HF_{TGAL}, that there may be an orientating function of macrophages, just as shown by Rosenthal *et al.*¹⁶ in macrophage-T cell interactions with insulin, although clearly many other mechanisms are possible.

There are thus macrophage *Ir* effects expressed at both the macrophage-T and macrophage-B interaction. It is not known how far the analogies may extend. For example, the inhibiting effects of anti-Ia antisera^{17,18} and the effects of the soluble Ia-containing macrophage mediator, genetically restricted macrophage factor (GRF)^{11,19,20}, suggest that macrophage *Ir* gene effects are caused by, or closely associated with Ia molecules. It will be of interest to ascertain the effects of anti-Ia antisera on macrophage-B cell interactions.

The concept of symmetry of macrophage-T and macrophage-B interactions suggests other predictions. For example, as there are genetic restrictions in macrophage-T interactions it is possible that there may also be such restrictions in the macrophage-B interaction. Such restrictions may help reconcile the apparent contradictions between *in vivo* experiments with helper factors, which have no genetic restrictions, within the species³ or outside it, as human helper factors work well in mice *in vitro* or *in vivo*²¹, and cell mixing experiments which often have genetic restrictions despite efforts to deplete alloreactive cells²².

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1. *Immun. Rev.*, Vol. 34 (ed. Møller, G.) (Munksgaard, Copenhagen, 1976).
2. Benacerraf, B. & McDevitt, H. O. *Science* **175**, 273-275 (1972).
3. Munro, A. J. & Taussig, M. J. *Nature* **256**, 103-106 (1975).
4. Kapp, J., Pierce, C. W. & Benacerraf, B. *J. exp. Med.* **140**, 172-184 (1974).
5. Feldmann, M. *Nature* **267**, 107 (1977).
6. Shevach, E. & Rosenthal, A. S. *J. exp. Med.* **138**, 1213-1229 (1973).
7. Schwartz, R. H., Yono, A. & Paul, W. E. in *Ir Genes and Io Antigens* (ed. McDevitt, H. O.) 297 (Academic, New York, 1978).
8. Miller, J. F. A. P., Vadas, M., Whitelaw, A., Gamble, J. & Bernard, C. *J. exp. Med.* **145**, 1623-1628 (1977).
9. Howie, S. & Feldmann, M. *Eur. J. Immun.* **7**, 417-421 (1977).
10. Feldmann, M. *J. exp. Med.* **136**, 737-760 (1972).
11. Erb, P. & Feldmann, M. *Nature* **254**, 352-354 (1975).
12. Kapp, J., Pierce, C. W. & Benacerraf, B. *J. exp. Med.* **138**, 1121-1132 (1973).
13. Dutton, R. W., McCarthy, M., Mishell, R. I. & Roudt, D. J. *Cell. Immun.* **1**, 196-202 (1970).
14. Yono, A., Schwartz, R. M. & Paul, W. E. *Eur. J. Immun.* (in the press).
15. Feldmann, M. & Nossal, G. J. V. *Transplantation* **13**, 3-34 (1972).
16. Rosenthal, A. S., Barcinski, M. & Blake, J. T. *Nature* **267**, 156-158 (1977).
17. Thomas, D., Yamashita, O. & Shevach, E. M. *J. Immun.* **119**, 223-226 (1977).
18. Schwartz, R. H. & Paul, W. E. *J. exp. Med.* **143**, 529 (1976).

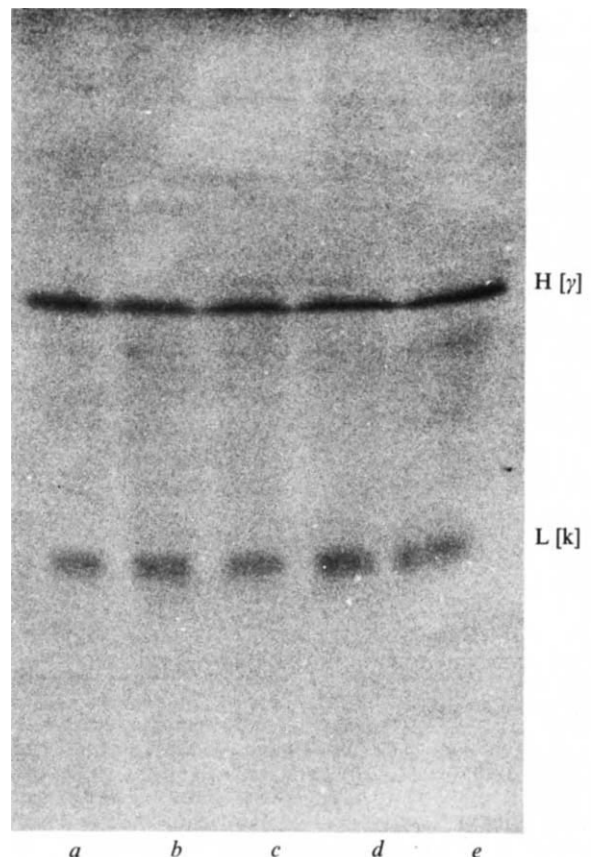
19. Erb, P., Meier, B., Kraus, D., Von Boehmer, H. & Feldmann, M. *Eur. J. Immun.* (in the press).
20. Erb, P. & Feldmann, M. *J. exp. Med.* **142**, 460-472 (1975).
21. Kantor, F. & Feldmann, M. *Clin. exp. Immun.* (submitted).
22. Sprent, J., Schwartz, R. H., Yono, A. & Paul, W. E. in *Ir Genes and Io Antigens* (ed. McDevitt, H. O.) 341 (Academic, New York, 1978).
23. Howie, S., Feldmann, M., Mozes, E. & Maurer, P. H. *Immunology* **32**, 297-299 (1977).
24. Taussig, M. J. *Nature* **248**, 234-235 (1974).
25. Feldmann, M., Greaves, M. F., Parker, D. C. & Rittenberg, M. B. *Eur. J. Immun.* **4**, 591-597 (1974).
26. Parish, C. R., Kirov, S. M., Bowern, N. & Blanden, R. V. *Eur. J. Immun.* **4**, 808 (1974).

Monoclonal antibodies against human lymphocyte antigens

MONOSPECIFIC antibodies produced by clones derived from appropriate hybridomas represent a new immunological tool of great analytical potential^{1,2}. In this report several antibodies against different surface antigens of human lymphocytes are described. They were obtained and isolated as individual monoclonal products after fusion of splenocytes from an immunised mouse with a mouse myeloma line.

The myeloma used was the 8-azaguanine resistant variant MOPC 21 (P3) NS1/1-Ag4-1 (ref. 3). Antibody forming cells (AFC) were induced in a female DBA 2/J mouse by one intravenous injection of 3.5×10^7 cells of a human lymphoblastoid line (HLL) obtained by Epstein-Barr (EB) virus transformation of blood from a HLA homozygous donor (line WT 52)⁴. The mouse spleen was taken for fusion 4 d later.

Fig. 1 Autoradiographs of sodium dodecyl sulphate-polyacrylamide gel electrophoresis, representing immunoglobulins in supernatants of selected clones. Slots a-e contain supernatants derived, respectively, from clones 2, 4, 6, 14, 18 of culture N 26. For antibody specificities see Table 1. The cultures were labelled overnight with ¹⁴C-leucine. Autoradiographs were exposed for 6 d. The band on the L region is due to overlapping of two k chains, one due to the antibody and the other synthesised by the myeloma and secreted after fusion³. The class and type of Ig were established by cytoplasmic immunofluorescence of the hybridoma cells with specific antisera.



Hybrids were obtained by the polyethylenglycol (PEG) method². The cell suspension was dispensed into 42 wells. After 13 d viable cells were observed in all wells growing in HAT selective medium⁵. Supernatants from these cultures were tested for complement-dependent lysis (Cdl) against the WT 52 line used for immunisation. Supernatants from seven cultures (N 10, 11, 16, 19, 24, 26, 32) were positive. None of these bound to mouse spleen cells or to human erythrocytes. Two cultures lost activity during expansion (N 11 and N 32).

Testing of 'Cdl-negative' cultures for noncytotoxic antibodies by a radioimmunoassay⁶ is in progress. Preliminary results suggest that about 30% of the wells produce antibodies that bind to the specific target⁷.

From each 'Cdl-positive' culture—with the exception of N 16—clones were derived in soft Agarose and then expanded in selective medium. The supernatants were tested for Cdl against a panel of 14 HLL derived from different donors. The patterns obtained are shown in Table 1.

Clones (Cl) derived from cultures N 10, N 19 and N 26 produced antibodies with the same specificities as the original primary culture. From culture N 24 clones with two distinct specificities were obtained.

Culture N 16 and clones from N 26 and N 24a were shown to produce antibodies against human β_2 -microglobulin (β_2 -m) by the following four criteria. (1) Cytotoxic activity was demonstrated against all peripheral blood lymphocytes from a panel of 30 donors and against all HLL tested, except the DAUDI line, which lacks membrane HLA (A, B, C) and β_2 -m (ref. 8). The Cdl had a titre of 1/64 against peripheral blood lymphocytes (PBL). (2) Cytotoxicity was completely blocked by addition of β_2 -m purified from human urine. Complete inhibition in the case of Cl 26/18 was obtained with the addition of β_2 -m $4 \mu\text{g ml}^{-1}$. A block of Cdl was also obtained by pre-treatment of cells with turkey antiserum to human β_2 -m (ref. 9). (3) Cytotoxicity of Cl 26/18 could be removed by passage of the supernatant through a column of β_2 -m-Sepharose¹⁰ and recovered by elution with 0.1 M acetic acid in 0.9% NaCl solution, followed by neutralisation of the eluate. (4) Nine clones from N 26 were tested and each was shown to bind radioactively labelled purified β_2 -m in the presence of ammonium sulphate at 50% saturation¹¹.

The reactivities of clones from culture N 19 and from culture N 24b were similar but not identical. In fact, they bound to a different number of HLL, and lines were observed which were N 19-positive and N 24-negative. This binding behaviour could not be correlated with known HLA types. Correlation with other characteristics of HLL, such as surface Ig or EB virus dependent antigens, is under study. Both types of antibodies

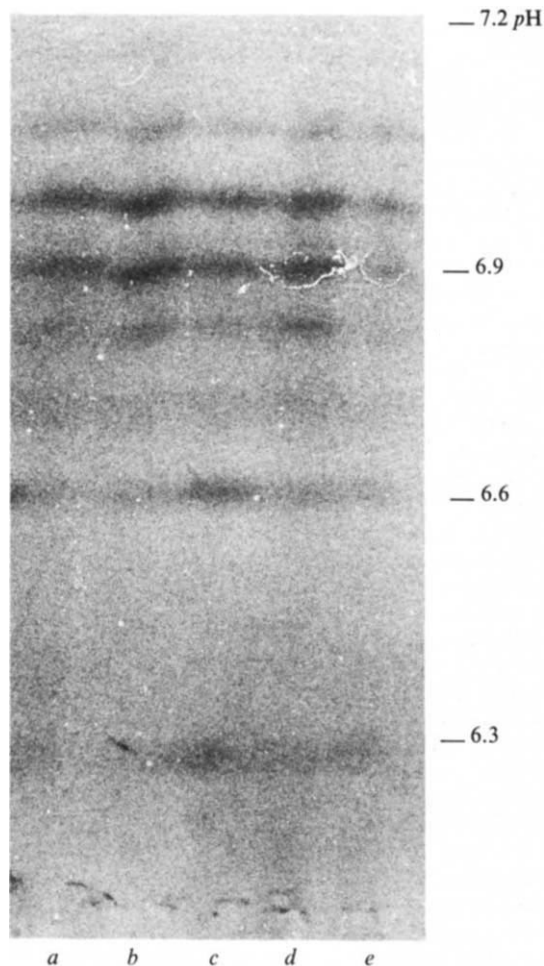


Fig. 2 Isoelectric focusing analysis¹⁴ of unreduced products in the supernatants of the five clones from culture N 26.

were not significantly active against PBL of any donor when tested by complement-dependent lysis (Table 1). However, by indirect immunofluorescence (IIF) using a rhodamine-labelled F(ab')₂ preparation from rabbit anti-mouse IgG, these antibodies stained a small number of PBL from the blood of every donor tested. All lymphocytes positive with these antibodies were shown to bear surface Ig (Ig⁺) by double staining using fluorescein-conjugated anti-human IgG. Among 30 different

Table 1 Patterns of reactivity of supernatants from representative clones

Primary culture	No. of clones tested	Human lymphoblastoid lines*												Peripheral blood lymphocytes				Specificity
		WT 52	1	2	3	6	8	4	5	9	11	7	10	MOLT 4	Daudi	Cdl‡	IIF§	
N 16	ND†	+	+	+	+	+	+	+	+	+	+	+	+	+	—	100	95	anti- β_2 -m
N 24a	1	+	+	+	+	+	+	+	+	+	+	+	+	+	—	100	95	
N 26	25	+	+	+	+	+	+	+	+	+	+	+	+	+	—	100	95	
N 19	30	+	+	+	+	+	+	+	+	+	+	—	—	—	+	<10	1-4	Subpopulation of B cells
N 24b	25	+	+	+	+	+	+	+	+	—	—	—	—	—	—	<10	0.1-7	
N 10	15	+	+	+	+	+	+	—	—	+	+	+	+	—	—	0-100	30-95	Subpopulation of PBL, some B and some T cells

Patterns of reactivity have been rearranged for emphasising similarities and differences. Note that at least three main patterns can be recognised. * WT 52: the B cell line used for immunisation—1 to 11: EB virus transformed HLL from other donors of the Basel-Torino panel, homozygous for HLA⁴; MOLT 4: a human T cell line¹⁷; note that it reacts only with anti- β_2 -m; Daudi: a Burkitt lymphoma line lacking membrane β_2 -m and HLA: A, B, C⁶. Each line was tested for reactivity with each monoclonal product by indirect immunofluorescence and by complement-dependent lysis (⁵¹Cr release¹⁸ and fluorochromasia¹⁹).

† Not done: cloning was technically unsuccessful, but chemical evidence (SDS-PAGE and isoelectric focusing) suggested antibody monoclonality.

‡ Complement-dependent lysis: by a dye exclusion test (NIH technique²⁰) % cells killed. <10 means below the sensitivity of the method.

§ Indirect immunofluorescence: % cells stained (see text).

blood samples tested, doubly positive cells varied between 1 and 40% of the total Ig⁺ lymphocytes.

Clones from culture N 10 again reacted only with some lines of the HLL panel and were shown both by Cdl and IIF to bind to 30–95% of PBL from all members of the donor panel. In this case, however, the positive population included Ig⁺ and Ig⁺ lymphocytes.

The Ig class of representative clones from N 19, N 24a, N 24b and N 26 was IgG. As spleen cells were taken for fusion 4 d after primary immunisation, the production of IgG antibodies in our experiments was unexpected (see, however, ref. 12). The cytotoxic activity in each case was found in the inclusion volume of a G-200 Sephadex column and eluted together with IgG. Each clone was found to bind to protein A-Sepharose. Supernatants of clones from N 19 and N 26, grown in the presence of ¹⁴C-leucine were submitted to polyacrylamide gel electrophoresis (PAGE) in reducing conditions¹³. In both cases homogenous protein bands corresponding in mobility to light chains and γ -chains were observed in autoradiographs of the gels. Monoclonality was further supported by isoelectric focusing¹⁴ (Figs 1 and 2).

These results confirm the ability of monoclonal antibodies arising from xeno-immunisation to recognise fine differences between surface antigens of normal and transformed human lymphocytes¹⁵. Thus, the mouse has a repertoire of antibody specificities which can detect well defined human species-specific antigens (as β_2 -m), differentiation antigens and histocompatibility (HLA) antigens (C. Barnstable *et al.*, unpublished). The production of antibodies to human β_2 -m by a number of different primary cultures confirms the unusual potency of this antigen in xeno-immunisation¹⁶.

The nature of the antigens carried on B lymphocytes which bind antibodies N 19 and N 24b is under study. It is possible that these represent an Ig subclass, that they are related to the EB virus or its receptor, or are previously undescribed differentiation antigens. In any case, they reveal a heterogeneity among HLL which may allow a more precise characterisation of the lines and of the PBL subclasses from which they derive.

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- K hler, G. & Milstein, C. *Eur. J. Immun.* **6**, 511–519 (1976).
- Galf r , G., Howe, S. C., Milstein, C., Butcher, G. W. & Howard, J. C. *Nature* **266**, 550–552 (1977).
- K hler, G., Howe, S. C. & Milstein, C. *Eur. J. Immun.* **6**, 292–295 (1976).
- Trucco, M., Galf r , G., De Marchi, M., Varetto, O. & Carbonara, A. O. *Tissue Antigens* **10**, 343–344 (1977).
- Littlefield, J. W. *Science* **145**, 709 (1964).
- Levy, R. & Dille, J. J. *Immun.* **119**, 387–393 (1977).
- Stocker, J. W., Ceppellini, R. & Trucco, M. M. in *Current Trends in Tumor Immunology* (ed. Gorini, S.) (Firenze: Fond. Menarini, in the press).
- Jones, E. A., Goodfellow, P. N., Bodmer, J. G. & Bodmer, W. F. *Nature* **256**, 650–653 (1975).
- Bernoco, D., *et al.* *Tissue Antigens* **8**, 253–260 (1976).
- Reisfeld, R. A., Allison, J. P., Ferrone, S., Pellegrino, M. A. & Poulik, M. D. *Transplant. Proc.* **8**, 173 (1976).
- Farr, R. S. *J. infect. Diseases* **103**, 239 (1958).
- Gearhart, P. J. *Nature* **269**, 812–813 (1977).
- Laemmli, U. K. & Faure, M. J. *molec. Biol.* **80**, 575–599 (1973).
- Cotton, R. G. H., Secher, D. S. & Milstein, C. *Eur. J. Immun.* **3**, 135–140 (1973).
- Lampson, L., Levy, R., Grumet, F. C., Ness, D. & Pious, D. *Nature* **271**, 461–462 (1978).
- Sanderson, A. *Nature* **269**, 414–415 (1977).
- Minowada, J., Ohnuma, T. & Miller, G. J. *Virol.* **6**, 699–701 (1970).
- Rogentine, G. N. *Histocompatibility Testing 1967*, 371–379 (Munksgaard, Copenhagen, 1967).
- Bodmer, W. F., Tripp, M. & Bodmer, J. G. *Histocompatibility Testing 1967*, 341–350 (Munksgaard, Copenhagen, 1967).
- Mottironi, V. D. & Terasaki, P. I. *Histocompatibility Testing 1970*, 301–307 (Munksgaard, Copenhagen, 1970).

Chido and Rodgers blood groups are distinct antigenic components of human complement C4

THE *HLA* complex consists of many closely linked genes which code for a variety of characters, particularly the expression of cell surface antigens found on lymphocytes (HLA-A, B, C, DR antigens) or erythrocytes (Chido and Rodgers blood groups) and polymorphic variation of certain complement components (Bf, C2, C4). We¹ have recently suggested that the polymorphism of the fourth component of human complement (C4), detected by immunofixation electrophoresis, is controlled by two genes linked to *HLA* and not by codominant alleles at a single locus as previously described^{2,3}. One C4 variant, designated F, is controlled by one locus with alleles determining the presence (*F*⁺) or absence (*F*⁰) of four fast-moving anodal bands of C4 while the second variant, S, is controlled by a second locus with alleles determining the presence (*S*⁺) or absence (*S*⁰) of four slow-moving cathodal bands. We noted that C4 F individuals homozygous for the *S*⁰ allele and lacking the four cathodal bands were always Chido (Ch^a) negative, whereas individuals homozygous for the *F*⁰ allele and lacking the four anodal bands of C4 (C4 S) were always Rodgers (Rg^a) negative. This intriguing observation led us to study the relationship of Chido and Rodgers red cell antigens, which are also found in serum and C4. We show here that Chido and Rodgers are distinct antigenic components of human C4, a finding which provides a biological explanation as to why these unique red cell antigens are controlled by genes of the *HLA* complex.

A panel of blood donors and family members were studied for their electrophoretic patterns of C4, Ch^a and Rg^a antigens and HLA-A, B and C lymphocyte antigens. The electrophoretic variants of C4 were determined according to the method of O'Neill *et al.*¹ and Ch^a and Rg^a typing was carried out using the plasma or serum inhibition test of anti-Ch^a or anti-Rg^a activity as previously described^{4,5}. Typing for HLA-A, B and C antigens was performed using the standard NIH two-stage, complement-dependent microcytotoxicity test⁶.

Figure 1a shows three electrophoretic patterns of bands of C4 detected in EDTA plasma by immunofixation electrophoresis. These consisted of four fast-moving anodal bands (F), four slow moving cathodal bands (S) or of all eight bands (FS). Individuals of the C4 F type had HLA-B antigens, B12, Bw35, B5 and B18 whereas those of the C4 S type were practically always HLA-B8 homozygous. These HLA-B antigen associations were of interest since it has been shown previously that lack of Ch^a[Ch(a[−])] is associated with HLA-B12 and Bw35^{7,8} and lack of Rg^a[Rg(a[−])] is associated with HLA-B8 homozygosity^{9–11}. From the results of Ch^a and Rg^a typing, it was striking to find that all individuals showing the C4 F variant (24 individuals) and lacking the four cathodal bands were clearly Ch(a[−]), Rg(a⁺) whereas all individuals showing the C4 S variant (13 individuals) were clearly Rg(a[−]), Ch(a⁺). Individuals expressing the C4 FS pattern (95 individuals) were Ch(a⁺), Rg(a⁺). Further evidence to support these observations was the finding that the donor of the anti-Ch^a antibody was HLA-B12/Bw35, showed the C4 F electrophoretic pattern and was Ch(a[−]), Rg(a⁺), whereas the donor of the anti-Rg^a antibody was HLA-B8 homozygous, C4 S and Rg(a[−]), Ch(a⁺). Figure 2 shows the families that illustrate the relationship of C4 electrophoretic variants, Ch^a and Rg^a antigens and antigens of the HLA-A and B series. Family Br. has two members that are C4 S, Rg(a[−]), Ch(a⁺) and HLA-B8 homozygous. Family Wa. has three members that are C4 F, Ch(a[−]), Rg(a⁺) and have HLA antigens B12 and Bw35. From the previous results we conclude that the *F*⁺ allele, determining the four anodal bands of C4, also controls the expression of the Rg^a antigen and that the *S*⁺ allele, determining the four cathodal bands of C4, controls the expression of the Ch^a antigen. Based on these observations, we predicted that individuals homozygous

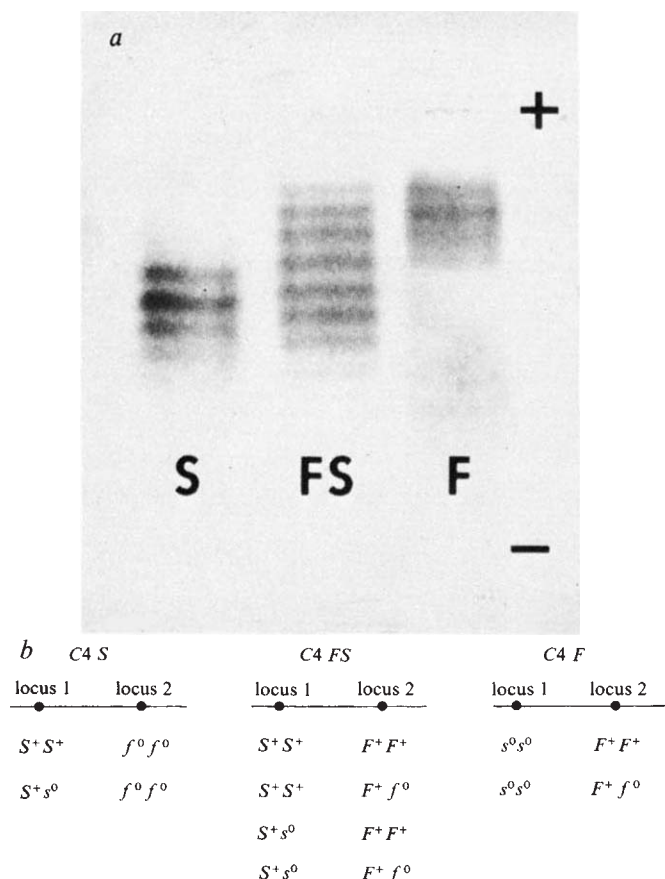


Fig. 1 *a*, C4 variants, C4 S (four cathodal bands), C4 FS (eight bands) and C4 F (four anodal bands) detected in EDTA plasma by agarose immunofixation electrophoresis in Tris-glycine-barbital buffer, pH 8.8. The C4 patterns were revealed by immunofixation with goat anti-human C4 (Meloy Laboratories). The phenotype frequencies of the C4 variants are C4 S=1%, C4 FS=92%, C4 F=7%. *b*, Possible genotypes for the C4 variants C4 S, C4 FS and C4 F.

deficient for C4 would show no bands of C4 on electrophoresis and be $Ch(a^-)$, $Rg(a^-)$. Serum was obtained from such an individual (provided by Dr Hans Grosse-Wilde) which lacked both functional and immunochemical C4 activity and which proved to be $Ch(a^-)$, $Rg(a^-)$. This is the first description of an individual lacking both Ch^a and Rg^a antigens.

Since anti- Ch^a or anti- Rg^a antibodies are formed in $Ch(a^-)$ or $Rg(a^-)$ individuals by transfusion of $Ch(a^+)$ or $Rg(a^+)$ plasma respectively, our results suggest that the C4 F and C4 S variants represent two distinct antigenic subclasses of C4, the Ch^a antigen being a component of the C4 S variant and the Rg^a antigen being a component of the C4 F variant.

If Ch^a and Rg^a are components of C4, then removal of C4 from serum should also result in removal of these antigens. To test this, normal human serum known to be $Ch(a^+)$, $Rg(a^+)$ and C4 FS was passed over an affinity chromatography column (Sephacrose 4B) to which goat anti-human C4 (Meloy Laboratories) was covalently bound. As a control, the same serum was passed over a similar affinity column to which goat anti-human IgG (Meloy Laboratories) had been similarly bound. The anti-C4 preparation showed a single precipitin arc on immunoelectrophoresis of normal human serum whereas no visible precipitin lines were formed on immunoelectrophoresis of C4 deficient human serum. To prevent activation of C4 during the chromatography, phenylmethylsulphonyl fluoride (PMSF) was added to the serum and the elution buffer (phosphate-buffered saline, pH 7.2) to a concentration of 10 mM. The results of these experiments are shown in Fig. 3. Similar protein elution profiles were obtained for both affinity columns. Each eluate fraction from both columns was tested for C4 activity (by

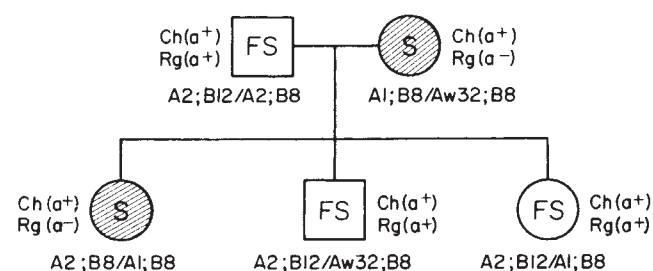
haemolytic and immunochemical assay) and for the presence of Ch^a and Rg^a antigens which were detected by the ability of those fractions containing Ch^a and Rg^a to inhibit anti- Ch^a and anti- Rg^a respectively and thus prevent the agglutination of $Ch(a^+)$ and $Rg(a^+)$ red cells using the anti-globulin technique. None of the fractions which eluted from the anti-C4 Sepharose had either C4 activity or showed the presence of Ch^a or Rg^a antigens, indicating that all the C4, Rg^a and Ch^a had been bound. C4 activity was detected in several of the fractions eluted from the anti-IgG Sepharose column. Ch^a and Rg^a antigens were present only in those fractions containing measurable C4 activity.

Further evidence showing that Ch^a and Rg^a are components of C4 is provided by experiments in which human C4, purified from pooled human serum (provided by Dr Irma Gigli) was able to replace whole serum in its ability to inhibit either anti- Ch^a or anti- Rg^a in the serum inhibition typing test for Ch^a or Rg^a antigens. Whereas both anti- Ch^a and anti- Rg^a were shown to agglutinate both $Ch(a^+)$ and $Rg(a^+)$ erythrocytes directly using the anti-globulin technique, prior incubation of the anti- Ch^a or anti- Rg^a with either purified C4 or, as a control, normal human serum (known to be $Ch(a^+)$, $Rg(a^+)$) resulted in the inhibition of both antisera and thus prevented the agglutination of $Ch(a^+)$ or $Rg(a^+)$ erythrocytes.

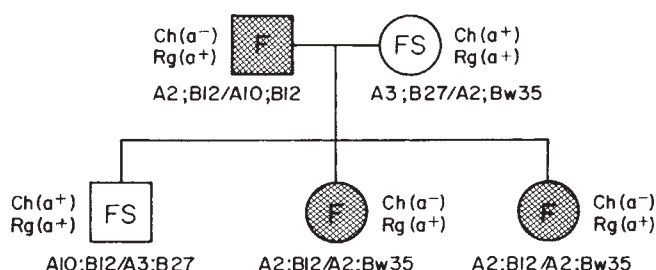
In the mouse, Démant *et al.*¹² have shown that the Ss-Slp region within the *H-2* complex controls the level of a serum complement component which has now been shown to be immunochemically and possibly functionally homologous to human C4 (refs 13–14). More recently evidence has been presented, that in the mouse the Ss and Slp proteins are two distinct antigenic subclasses of C4 (ref. 15) with possibly distinct functional properties¹⁶. Our results indicate that the C4 FS electrophoretic variant of C4 is composed of two antigeni-

Fig. 2 HLA genotypes, Ch^a and Rg^a blood group typing and C4 variants (F, FS and S) in families Br. and Wa. In family Br. the possible genotypes of the parents would be: father (C4 FS) $F^+ S^+ / f^0 S^+$ (or $F^+ s^0 / f^0 S^+$); mother (C4 S) $f^0 S^+ / f^0 S^+$ and therefore the C4 S child would be $f^0 S^+ / f^0 S^+$. In family Wa. the possible genotypes of the parents would be: father (C4 F) $F^+ s^0 / F^+ s^0$; mother (C4 FS) $F^+ S^+ / F^+ s^0$ (or $F^+ s^0 / f^0 S^+$), so the two C4 F children would be $F^+ s^0 / F^+ s^0$.

Family Br (Rodgers negative)



Family Wa (Chido negative)



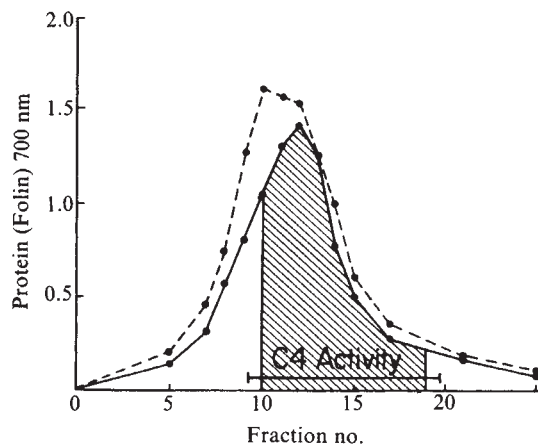


Fig. 3 Elution profiles of serum proteins from 1, a 1×15 cm column of anti-C4 Sepharose (broken line) and 2, a 1×15 cm column of anti-IgG Sepharose (solid line). 0.5 ml samples of human serum were applied to each column and then eluted with phosphate-buffered saline, pH 7.2 containing 10 mM PMSF. C4, Ch^a and Rg^a were not detected in any fraction from column 1. From column 2, Ch^a and Rg^a antigens were detected only in those fractions containing C4 and are represented by the shaded area. Fraction 14 was shown to contain the highest concentration of C4 corresponding to 33% of the C4 applied.

cally distinct subclasses of C4, the Ch^a antigen distinguishing a component of the C4 S variant and the Rg^a antigen distinguishing a component of the C4 F variant. In the two gene model for the control of the C4 variants, C4 S and C4 F would be isotypes both of which we have shown to have haemolytic activity *in vitro*. Allotypic forms of C4 would be determined by the presence (F^+) or absence (f^0/f^0) of the four anodal bands, simultaneously characterised by the presence or absence of the Ch^a antigen. Similarly the presence (S^+) or absence (s^0/s^0) of the four cathodal bands would correspond to the presence or absence of the Rg^a antigen. The possible genotypes for C4 S, C4 FS and C4 F for this two gene model are given in Fig. 1b. The correspondence between the C4 electrophoretic variants and Chido and Rodgers implies that these red cell antigens are controlled by two distinct genes. Previous genetic studies on the relationship between Ch^a and Rg^a indicate that they are not alleles^{8,9}. Our studies not only qualify this but also explain why Ch(a⁻), Rg(a⁻) individuals have not been identified previously. Such individuals will only be found, as we have shown, when studying C4 homozygous deficient patients and this condition is very rare^{17,18}. The two genes coding for Ch^a and Rg^a are both closely linked to the *HLA-B* locus^{8,9} which explains the genetic linkage disequilibrium between Rg(a⁻) and Ch(a⁻) and *HLA-B8*.

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- O'Neill, G. J., Yang, S. Y. & Dupont, B. *Proc. natn. Acad. Sci. U.S.A.* (in the press).
- Teisberg, P., Åkesson, I., Olaisen, B., Gedde-Dahl, T., Jr & Thorsby, E. *Nature* **264**, 253–254 (1976).

- Teisberg, P., Olaisen, B., Jonassen, R., Gedde-Dahl, T., Jr & Thorsby, E. *J. exp. Med.* **146**, 1380–1389 (1977).
- Middleton, J. & Crookston, M. C. *Vox Sang.* **23**, 256–261 (1972).
- Longster, G. & Giles, C. M. *Vox Sang.* **30**, 175–180 (1976).
- Mittal, K. K., Hasegawa, T., Ting, A., Mickey, M. R. & Terasaki, P. I. in *Histocompatibility Testing 1972* (eds Dausset, J. & Colombani, J.) 187–195 (Munksgaard, Copenhagen, 1973).
- Middleton, J. *et al. Tissue Antigens* **4**, 366–373 (1974).
- Gedde-Dahl, T., Jr, Giles, C. M., Thorsby, E., Olaisen, B. & Robson, E. B. in *Human Gene Mapping 3: Birth Defects, Original Ser.* **12**, 307–310 (Karger, Basel, 1976).
- Giles, C. M. *et al. Tissue Antigens* **8**, 143–149 (1976).
- Gedde-Dahl, T., Jr & Robson, E. B. in *Human Gene Mapping 3: Birth Defects, Original Ser.* **12**, 311–312 (Karger, Basel, 1976).
- James, J., Stiles, P., Boyce, F. & Wright, J. *Vox Sang.* **30**, 214–216 (1976).
- Démant, P., Capková, J., Hinzová, E. & Voráčová, B. *Proc. natn. Acad. Sci. U.S.A.* **70**, 863–864 (1973).
- Meo, T., Krasteff, T. & Shreffler, D. C. *Proc. natn. Acad. Sci. U.S.A.* **72**, 4536–4540 (1975).
- Lachmann, P. J., Grennan, D., Martin, A. & Démant, P. *Nature* **258**, 242–243 (1975).
- Roos, M. H., Atkinson, J. P. & Shreffler, D. C. *J. Immun.* **120**, 1794 (1978).
- Ferreira, A., Takahashi, M. & Nussenzweig, V. *J. exp. Med.* **146**, 1001–1018 (1977).
- Hauptmann, G., Grosshans, E. & Heid, E. *Ann. Dermatol. Syphiligr. (Paris)* **101**, 479–496 (1974).
- Ochs, H. D. *et al. N. Engl. J. Med.* **296**, 470–475 (1977).

Biosynthesis of prostacyclin in rat liver endothelial cells and its control by prostaglandin E₂

PROSTACYCLIN (PGI₂), a member of the prostaglandin family first identified in blood vessel preparations^{1–3}, has been shown to be produced by the heart^{4,5} and placenta⁶. More recently, PGI₂ biosynthesis has been demonstrated in cultured endothelial cells incubated with arachidonate. Thus, it seems that endothelial cells not only in the blood vessels of the systemic circulation, but, as expected, also in the organ vasculature, are capable of producing PGI₂. Studies of the mechanism of action of prostaglandins in the liver have indicated an action of PGE₂ exerted exclusively on cyclic AMP levels of non-parenchymal (endothelial-rich) cells⁹. It was proposed that PGE₂ may behave as an intercellular messenger that, once produced by parenchymal cells, would act on sinusoidal cells by increasing cyclic AMP levels^{10,11}. We report here that PGE₂ inhibits PGI₂ biosynthesis in rat liver endothelial cells, probably by increasing cyclic AMP levels.

PGI₂ was identified and quantitated by assaying the effect of endothelial-rich cell suspensions (EC)⁹ or supernatant aliquots on platelet aggregation measured with a Born-type two-channel aggregometer. Unless otherwise stated, EC were added to 0.5 ml human platelet-rich plasma (PRP) 5 min before 1.5 mM sodium arachidonate (SA) induced aggregation. Cyclic AMP levels and proteins were assayed as previously reported^{9,12}. Addition of aliquots of EC suspensions to PRP produced a dose- and time-dependent inhibition of arachidonate-induced aggregation (Fig. 1). When aggregation was completely inhibited, and the sample was allowed to stand at 37°C for 11 min, addition of 1.5 mM arachidonate caused aggregation. These results can be explained by assuming the formation of a PGI₂-like prostaglandin by rat liver EC. PGI₂ is known to be a potent inhibitor of aggregation; however, it is unstable at pH 7.5 and is converted to 6-keto-PGF_{1α} within 10 min at 37°C (refs 2, 3). Boiled EC or parenchymal cells (50 μg protein) were ineffective on arachidonate-induced aggregation. Rat liver microsomes were at least 10-fold less effective than EC homogenates (see also ref. 13). By standard plots similar to that shown in Fig. 1, it was estimated that after 5 min at 37°C EC produced 18.9 ± 4.6 ng PGI₂ per mg protein (mean ± s.e.m., $n = 5$), a figure which agrees with that reported by Bunting *et al.*³ for blood vessel rings. The formation of PGI₂ in our preparations was confirmed by the chromatographic identification of labelled 6-keto-PGF_{1α} in extracts of EC homogenates incubated with labelled arachidonate (unpublished data). 6-keto-PGF_{1α} biosynthesis was inhibited by tranylcypromine (0.5 mg per ml).

It has been found that PGE₁ or PGE₂ markedly increased cyclic AMP levels of EC⁹. As PGE₂ is apparently formed

mainly in liver parenchymal cells¹¹, these results suggest that parenchymal cells send messages to EC in the form PGE₂ which, by increasing cyclic AMP, regulates their metabolism. Our results suggest that PGE₂ inhibits the formation of PGI₂ in EC. Figure 2 shows that PGE₂ inhibited PGI₂ formation in EC suspensions in a dose- and time-dependent way. The effect on platelet aggregation is consistent with the increase of cyclic AMP levels elicited by PGE₂. Dibutyl-cyclic AMP had an effect similar to that of PGE₂. Inhibitory effects of cyclic AMP on prostaglandin biosynthesis in platelets have been reported^{14,15}.

Thus, in the liver, prostaglandins seem able to modulate platelet aggregation as well as the flow of the blood in the sinusoids. In the rat, prostacyclin synthesis inhibition probably contributes to the pressor effect of PGE₂ (ref. 16).

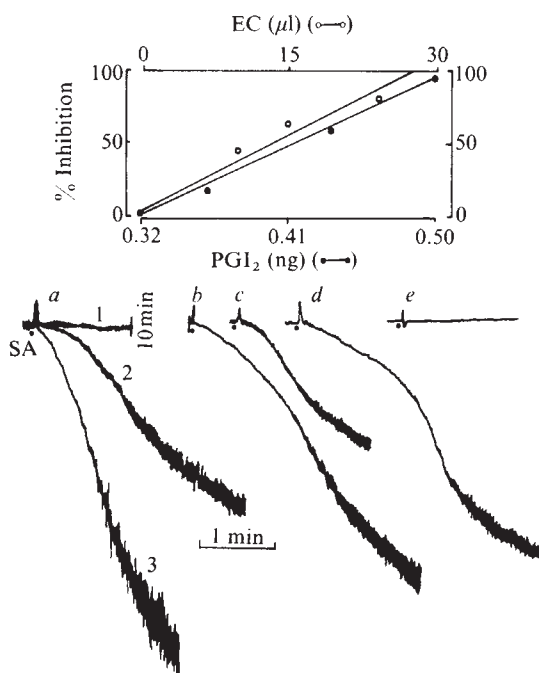


Fig. 1 The effect of endothelial-rich cell suspensions (EC) on the aggregation of human platelet-rich plasma (PRP). PRP was prepared by centrifuging venous blood (9 volumes: 1 volume of 3.8% sodium citrate) at 200g for 15 min; platelet poor plasma (PPP) was obtained by centrifuging PRP at 800g for 10 min. EC were obtained by perfusing rat liver with collagenase as previously described^{9,12} and by centrifuging the 40g supernatant at 1,000g for 10 min. The sediment suspended in Krebs-Ringer bicarbonate buffer (pH 7.4) was filtered and washed twice. The white pellets above a reddish sediment were suspended in buffer to give 2.1–2.4 mg protein per ml. This suspension contained endothelial cells and Kupffer cells in a 5:2 ratio and few blood cells. PRP was incubated for 5 min with EC suspension (22–55 μg protein) or for 2 min with PGI₂ and aggregation was induced with 1.5 mM sodium arachidonate (SA). Arachidonic acid (Sigma, type 1) was dissolved in ethanol and diluted before use with 2% Na₂CO₃ in 0.9% NaCl. Synthetic prostacyclin sodium salt (Upjohn) was dissolved in 0.05 M Tris-HCl, pH 9.32, and diluted with buffer before use. Endogenous formation of PGI₂ in EC was calculated by the standard plot shown. The aggregometer was calibrated at 100% aggregation with PPP containing appropriate amounts of EC. Each point is the mean of two observations. *a*, 3, control; *2*, EC (95 μg protein) not preincubated with PRP; *1*, EC preincubated for 5 min. *b*, as *1* but after 10 min at 37°C; *c*, EC (44 μg protein) incubated for 10 min in buffer, centrifuged and resuspended in buffer; *d*, as (*c*) but in the presence of 5 μM indomethacin; *e*, EC (100 μl) were incubated with 1.2 mM SA (5 min at 37°C) and centrifuged at 1,000g; the sediment suspended in buffer was incubated for 5 min at 37°C; 50 μl of supernatant was added to PRP.

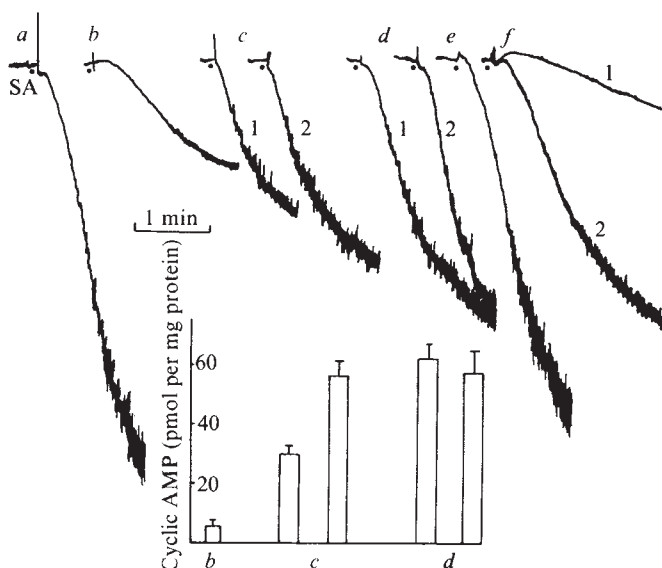


Fig. 2 Inhibitory effect of PGE₂ on prostacyclin biosynthesis in EC. Aliquots (15 μl, 40 μg protein) of EC were incubated at 37°C in aggregometer cuvettes in the absence (*b*) or presence of 0.15 μg (*c*, 1) or 0.3 μg (*c*, 2) PGE₂. The volume was brought to 0.1 ml with buffer; 5 min later, 0.5 ml PRP was added and after 5 min aggregation was induced with 1.5 mM SA. *d*, 1 and 2, as in *c* but preincubation with PGE₂ was for 10 min at 37°C. *f*, 2, EC (47 μg protein) were incubated (5 min at 37°C) with 1.5 mM dibutyl-cyclic AMP before addition of PRP (0.3 mM dibutyl-cyclic AMP added to PRP does not influence SA-induced aggregation); *f*, 1, control containing only EC. *a*, SA-induced aggregation; *e*, in the presence of PGE₂ (0.3 μg). Bars represent levels of cyclic AMP assayed on EC (350 μg protein) incubated in the absence (*b*) or presence (*c*, *d*) of PGE₂, in the same conditions described above. Final volume was 0.3 ml; data are means ± s.e.m. of three assays.

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1. Pace-Asciak, C. & Wolfe, L. S. *Biochemistry* **10**, 3657–3664 (1971).
2. Moncada, S., Gryglewski, R., Bunting, S. & Vane, J. R. *Nature* **263**, 663–665 (1976).
3. Bunting, S., Gryglewski, R., Moncada, S. & Vane, J. R. *Prostaglandins* **12**, 897–913 (1976).
4. Isakson P. C. *et al. Proc. natn. Acad. Sci. U.S.A.* **74**, 101–105 (1977).
5. De Deckere, E. A. M., Nugteren, D. H. & Ten Hoor, F. *Nature* **268**, 160–163 (1977).
6. Myatt, L. & Elder, M. G. *Nature* **268**, 159–160 (1977).
7. Weksler, B. B., Marcus, A. J. & Jaffe, E. A. *Proc. natn. Acad. Sci. U.S.A.* **74**, 3922–3926 (1977).
8. MacIntyre, D. E., Pearson, J. D. & Gordon, J. L. *Nature* **271**, 549–551 (1978).
9. Ferretti, E., Biondi, C. & Tomasi, V. *FEBS Lett.* **69**, 70–74 (1976).
10. Tomasi, V. *Expl Cell Biol.* **44**, 260–277 (1976).
11. Bartolini, G., Meringolo, C., Orlandi, M. & Tomasi, V. *Biochim. biophys. Acta* (in the press).
12. Barnabei, O., Leghissa, G. & Tomasi, V. *Biochim. biophys. Acta* **362**, 316–325 (1974).
13. Moncada, S., Gryglewski, R., Bunting, S. & Vane, J. R. *Prostaglandins* **12**, 715–737 (1976).
14. Malmsten, C., Granstrom, E. & Samuelsson, B. *Biochem. biophys. Res. Commun.* **68**, 569–576 (1976).
15. Lapetina, E. G. *et al. Biochem. biophys. Res. Commun.* **76**, 825–835 (1977).
16. Malik, K. U. & McGiff, J. C. *Circulation Res.* **36**, 599–609 (1975).

Cyclic AMP as a mitogenic signal for cultured rat Schwann cells

THE possible role of intracellular cyclic AMP in the control of cell division is the subject of much controversial literature (reviewed in refs 1, 2). In most such studies fibroblastic cell lines have been used, and various manipulations which raise intracellular concentration of cyclic AMP have been reported to inhibit cell proliferation¹. Here we show that highly purified Schwann cells in dissociated cell cultures of newborn rat nerve, which divide infrequently in 10% serum, are stimulated to divide when exposed to cholera toxin (an irreversible activator of adenyl cyclase activity^{3,4}), 3-isobutyl-L-methyl-xanthine (IBMX, a 3',5'-cyclic nucleotide phosphodiesterase inhibitor⁵), or dibutyryl cyclic AMP, all of which would be expected to increase intracellular cyclic AMP levels.

We have previously shown that the two major cell types in dissociated cell cultures of newborn rat sciatic nerve, fibroblasts and Schwann cells, can be distinguished by surface antigenic markers⁶: the Thy 1.1 antigen can be detected only on the fibroblasts, and the rat neural antigen-1 (Ran-1), defined by a mouse antiserum against a chemically induced rat neural tumour⁷, is only on the Schwann cells. By combining immunofluorescence with autoradiography following exposure of the cells to ³H-thymidine, it was found that the majority of fibroblasts in cultures maintained in 10% serum were rapidly dividing while the majority of Schwann cells were not⁶. Thus, by treating primary cultures of sciatic nerve for 48 h with the antimitotic drug cytosine arabinoside (10^{-5} M), the majority of fibroblasts could be killed while most Schwann cells survived (unpublished). After 1 week in culture, these cytosine arabinoside-treated primary cells were removed from the culture flasks with trypsin and treated once or twice with mouse anti-Thy 1.1 antiserum (1:8) and rabbit complement (1:5) to eliminate the remaining fibroblasts. Such secondary cells consisted of more than 99.5% Ran-1⁺ Schwann cells and less than 0.5% Thy 1.1⁺ fibroblasts (unpublished).

These secondary Schwann cells divided infrequently in Dulbecco's modified Eagle's medium (MEM) containing 10% heat-inactivated foetal calf serum (FCS), doubling approximately every 7–8 days even when the medium was changed every 3 days (Fig. 1). The shape of the growth curve suggests a relatively homogeneous population of infrequently dividing cells rather than a mixture of non-dividing and rapidly dividing cells. When cholera toxin (Schwarz-Mann) was added to the medium, the cell number increased rapidly, doubling about every 48 h until reaching confluence at $60\text{--}80 \times 10^3$ cells per cm^2 (Fig. 1). If the initial plating density was low, Schwann cells continued to proliferate rapidly for more than 2 weeks, either in the continuous presence of cholera toxin (Fig. 1) or after a 3-day exposure to the toxin (100 ng ml^{-1}) (data not shown). Dibutyryl cyclic AMP (10^{-5} M) was also mitogenic for Schwann cells, although to a lesser extent than cholera toxin (Fig. 1).

The mitogenic effect of cholera toxin and dibutyryl cyclic AMP was also demonstrated by autoradiography. Secondary Schwann cells were exposed to these agents for a total of 72 h and ³H-thymidine was added for the last 24 h of the culture. In a typical experiment (Table 1), 27% of Schwann cells were labelled in control cultures; this proportion was increased to 91% by cholera toxin (5 ng ml^{-1}) and 64% by dibutyryl cyclic AMP (10^{-5} M).

To study Schwann cell proliferation more readily, we adapted an assay commonly used to study mitogen-induced lymphocyte proliferation, the incorporation of [5-¹²⁵I]2-deoxyuridine (¹²⁵I-UdR) into DNA in cells growing in microwells. Purified secondary Schwann cells plated in microwells for 1–3 days were exposed to various agents for 24, 48 or 72 h and ¹²⁵I-UdR was added for the last 16–18 h. The cells were then collected and counted in a γ counter.

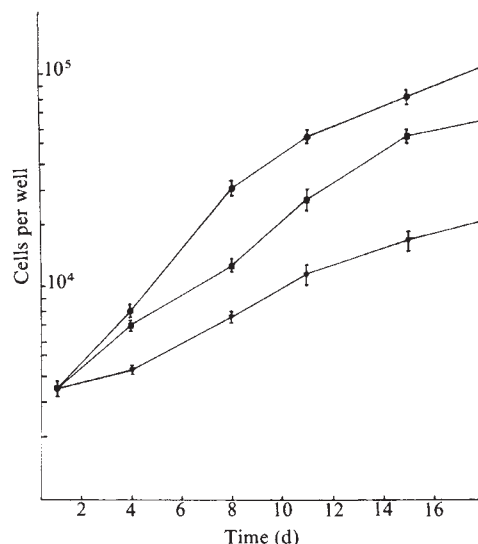


Fig. 1 Effect of cholera toxin and dibutyryl cyclic AMP on Schwann cell proliferation. 5×10^3 secondary Schwann cells (prepared as in text) were plated in Linbro dishes (Flow, $24 \times 1.77 \text{ cm}^2$ wells) in 500 μl of MEM and 10% FCS. On day 0 cholera toxin (5 ng ml^{-1}) or dibutyryl-cyclic AMP (Sigma, grade II-A, 10^{-5} M) was added. The medium was changed and new reagent added every 3 d. Cells were removed from the wells with trypsin and EDTA and counted in a haemocytometer. The results are expressed as means of three wells $\pm$ s.d. control: ▼; cholera toxin, ●; dibutyryl cyclic AMP, ■.

Cholera toxin, dibutyryl cyclic AMP and the phosphodiesterase inhibitor IBMX all significantly increased ¹²⁵I-UdR incorporation compared to controls (Table 2); the effect could be seen within 24 h but was greater at 48 and 72 h. Cholera toxin was active at 0.01 pg ml^{-1} , but choleraenoid, the biologically inactive derivative of cholera toxin^{3,4} which binds equally well to the same cell surface ganglioside, GM₁ (ref. 4), was without effect when used at concentrations up to 500 ng ml^{-1} (Table 2); by indirect immunofluorescence choleraenoid could be shown to bind to Schwann cells in a manner not appreciably different from cholera toxin (see below). Dibutyryl cyclic AMP was mitogenic at 10^{-5} – 10^{-4} M but inhibited ¹²⁵I-UdR incorporation at 10^{-3} M (Table 2). Dibutyryl cyclic GMP was not mitogenic (Table 2), nor did it inhibit the mitogenic effect of dibutyryl cyclic AMP. Cyclic AMP, 5'-AMP, sodium butyrate, prostaglandin E₁ and isoprenaline were not mitogenic over a wide concentration range (data not shown). IBMX was weakly mitogenic and when used at 3×10^{-5} M it reproducibly potentiated the effect of dibutyryl

Table 1 Effect of cholera toxin and dibutyryl cyclic AMP on the proportion of Schwann cells labelled with ³H-thymidine

Stimulant	% Of Schwann cells labelled
0	27.3 $\pm$ 0.6
Cholera toxin	91 $\pm$ 2
Dibutyryl cyclic AMP	64 $\pm$ 1

On day 0 secondary Schwann cells were plated on glass coverslips in wells of a Linbro plate (10,000 cells per coverslip) in 500 μl MEM and 10% FCS. Cholera toxin (5 ng ml^{-1}) or dibutyryl-cyclic AMP (10^{-5} M) was added on day 1 and ³H-thymidine ($2 \mu\text{Ci ml}^{-1}$) on day 3. On day 4 coverslips were washed, fixed in acid-alcohol, washed and coated with Kodak NTB2 emulsion. After 2 d at 4°C in the dark, the coverslips were developed and stained with Giemsa stain. Cells with >10 grains over their nucleus were scored as labelled. Results expressed as means $\pm$ s.d. of three cultures.

cyclic AMP (Table 2). Cholera toxin, dibutyryl cyclic AMP and IBMX were all mitogenic for Schwann cells cultured in 10 or 20% FCS but not in $\leq 1\%$ FCS.

Our results strongly suggest that elevated levels of intracellular cyclic AMP stimulate normal Schwann cells to proliferate *in vitro*. The only known effect of cholera toxin on mammalian cells is to increase the activity of adenylyl cyclase and thus raise the intracellular concentration of cyclic AMP (refs 3, 4). As more than 50% of Schwann cells in our secondary cultures could be shown by indirect immunofluorescence to bind large amounts of cholera toxin (unpublished observations) and as the cultures contained less than 0.5% (and sometimes less than 0.1%) non-Schwann cells, it is highly likely that the prolonged mitogenic effect of cholera toxin was due to the fact that it increased the levels of cyclic AMP within Schwann cells. The mitogenic activity of dibutyryl cyclic AMP and IBMX and the lack of effect of cholera toxin support this view. In preliminary experiments, we have directly demonstrated a sustained

increase in cyclic AMP levels in purified Schwann cells treated with cholera toxin or dibutyryl cyclic AMP but not with cholera toxin, prostaglandin E_1 or isoprenaline (E. Abney, M.C.R., A.H.S., J.P.B., unpublished). Although there have been previous reports suggesting a role for cyclic AMP in promoting proliferation of some normal cells⁸⁻¹² or cell lines¹³, to our knowledge, this is the first demonstration that an increase in intracellular cyclic AMP is mitogenic for a purified population of normal cells; where unpurified populations have been studied⁸⁻¹¹ or experiments have been done *in vivo*¹², one cannot exclude the possibility that cyclic AMP is acting indirectly by inducing the secretion of a mitogen. In the case of normal and transformed cell lines, the great majority of studies have associated increased levels of intracellular cyclic AMP with growth inhibition¹. Although some of these differences may be due to differences between normal cells and cell lines, it seems likely that at least some of them reflect genuine differences between cell types, as is well known for other cyclic AMP-mediated effects. It is interesting, however, that cholera toxin inhibits rather than stimulates ¹²⁵I-UdR incorporation in the cloned Schwannoma cell line RN2 (unpublished observations).

In rodent embryos Schwann cell proliferation is an important part of peripheral nerve development¹⁴. However, at birth more than 70% of Schwann cells in rodent sciatic nerve have stopped dividing¹⁵, and in adults the proportion of dividing Schwann cells is less than 1% (refs 15, 16). Adult Schwann cells are stimulated to divide when a nerve is injured^{17,18}, and this proliferation is thought to be important in nerve regeneration¹⁹; moreover, repeated injury can lead to a marked overproduction of Schwann cells²⁰. Schwann cells in culture have been reported to be induced to divide by contact with sensory²¹ or sympathetic²² neurones in the same culture dish. Our results raise the possibility that one of the intracellular signals for division in Schwann cells during neurogenesis or nerve regeneration or on contact with neurones *in vitro* may be a sustained elevation in intracellular cyclic AMP. It has been reported that cyclic AMP levels are increased below and above the point of a crush injury to rabbit sciatic nerve within 6 and 24 h, respectively, of the injury²³, thus preceding the characteristic wave of Schwann cell proliferation¹⁸.

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- Pastan, I. H., Johnson, G. S. & Anderson, W. B. *A. Rev. Biochem.* **44**, 291-522 (1975).
- Chlapowski, F. J., Kelly, L. A. & Butcher, R. W. *Adv. Cyclic Nucleotide Res.* **6**, 245-239 (1975).
- Finkelstein, R. A. *CRC Crit. Rev. Microbiol.* **2**, 553-575 (1973).
- Bennett, V. & Cuatrecasas, P. in *The Specificity and Action of Animal, Bacterial and Plant Toxins* 1, (ed. Cuatrecasas, P.) 3-66 (1977).
- Matsuzawa, H. & Nirenberg, M. *Proc. natn. Acad. Sci. U.S.A.* **72**, 3472-3476 (1975).
- Brookes, J. P., Fields, K. F. & Raff, M. C. *Nature* **266**, 364-366 (1977).
- Fields, K. F., Gosling, C., Megson, M. & Stern, P. L. *Proc. natn. Acad. Sci. U.S.A.* **72**, 1296-1300 (1975).
- Hovi, T. & Vaheri, A. *Nature new Biol.* **245**, 175-177 (1973).
- Creighton, M. O. & Trevithick, J. R. *Nature* **249**, 767-768 (1974).
- Whitfield, F. F. *et al. In Vitro* **12**, 1-18 (1976).
- Filosa, S., Pictet, R. & Rutter, W. J. *Nature* **257**, 702-705 (1975).
- Short, J., Tsukada, K., Rudert, W. A. & Lieberman, I. *J. biol. Chem.* **250**, 3602-3606 (1975).
- Pawelek, J. Halaban, R. & Christie, G. *Nature* **258**, 539-540 (1975).
- Peters, A. & Muir, A. R. *J. exp. Physiol.* **44**, 117-130 (1959).
- Asbury, A. K. *J. Cell Biol.* **34**, 735-743 (1967).
- Friede, R. L. & Johnstone, M. A. *Acta Neuropath.* **7**, 218-231 (1967).
- Abercrombie, M. & Johnson, J. L. *J. Anat.* **80**, 37-50 (1946).
- Bradley, W. G. & Asbury, A. K. *Expl Neurol.* **26**, 275-282 (1970).
- Causey, G. *The Cell of Schwann* (Livingstone, London, 1960).
- Thomas, P. K. *J. Anat.* **106**, 463-470 (1970).
- Wood, P. M. & Bunge, R. P. *Nature* **256**, 662-664 (1975).
- McCarthy, K. D. & Partlow, L. M. *Brain Res.* **114**, 416-426 (1976).
- Appenzeller, O. & Palmer, G. *Brain Res.* **42**, 521-524 (1972).

Table 2 Effect of cholera toxin, cyclic nucleotides and IBMX on ¹²⁵I-UdR incorporation into Schwann cells

Stimulant	Concentration (ml ⁻¹)	Schwann cell proliferation ¹²⁵ I-UdR incorporation (c.p.m.)*	Stimulation ratio†
Expt 1			
0	—	290 ± 74	—
Cholera toxin	0.1 pg	720 ± 164	2.5
Cholera toxin	1 pg	818 ± 24	2.8
Cholera toxin	10 pg	1,068 ± 129	3.7
Cholera toxin	100 pg	1,385 ± 136	4.8
Cholera toxin	1 ng	1,860 ± 108	6.4
Cholera toxin	10 ng	2,023 ± 145	6.9
Expt 2			
0	—	831 ± 92	—
Cholera toxin	1 ng	3,267 ± 47	3.9
Cholera toxin	1 ng	701 ± 47	<1
Cholera toxin	10 ng	811 ± 34	<1
Cholera toxin	100 ng	1,011 ± 14	1.2
Cholera toxin	500 ng	1,035 ± 87	1.2
Expt 3			
0	—	770 ± 50	—
dbc AMP‡	10 ⁻⁶ M	977 ± 53	1.3
dbc AMP	10 ⁻⁵ M	1,950 ± 40	2.5
dbc AMP	10 ⁻⁴ M	1,171 ± 96	1.5
IBMX	3 × 10 ⁻⁶ M	948 ± 60	1.2
IBMX	3 × 10 ⁻⁵ M	1,510 ± 107	1.9
IBMX	3 × 10 ⁻⁴ M	674 ± 24	<1
IBMX§ + dbc AMP	10 ⁻⁶ M	1,722 ± 50	2.2
IBMX + dbc AMP	10 ⁻⁵ M	1,801 ± 80	2.3
IBMX + dbc AMP	10 ⁻⁴ M	700 ± 6	<1
Expt 4			
0	—	1,131 ± 177	—
dbc AMP	10 ⁻⁶ M	2,115 ± 132	1.9
dbc AMP	10 ⁻⁵ M	3,569 ± 214	3.2
dbc AMP	5 × 10 ⁻⁵ M	3,467 ± 520	3.1
dbc AMP	10 ⁻⁴ M	1,503 ± 201	1.3
dbc AMP	10 ⁻³ M	530 ± 104	<1
dbc GMP	10 ⁻⁶ M	1,263 ± 121	1.1
dbc GMP	10 ⁻⁵ M	1,269 ± 59	1.1
dbc GMP	10 ⁻⁴ M	1,237 ± 131	1.1

Secondary Schwann cells were plated in Linbro dishes (96 × 1.13 cm² flat-bottom wells; 10,000 cells per well) in 100 μl MEM and 10% FCS. After 1-3 d, various stimulants were added for 48 (experiments 2-4) or 72 (experiment 1) h, and ¹²⁵I-UdR (2 μCi ml⁻¹) was added for the final 16-18 h. The cells were collected using a multiple sample harvester and counted in a Wallac GTL γ counter.

* Results expressed as means ± s.d. of three cultures.

† Stimulation ratios = ¹²⁵I-UdR incorporation with stimulant/¹²⁵I-UdR incorporation in control.

‡ Dibutyryl cyclic AMP.

§ 3 × 10⁻⁵ M.

Is somatostatin an excitatory transmitter in the hippocampus?

THE presence of growth hormone release inhibiting factor, somatostatin, in extrahypothalamic sites in the brain^{1,2} suggests that, in common with several other biologically active peptides, it may be a neurotransmitter or modulator of central nervous system (CNS) neuronal activity in addition to its neuroendocrine role. Direct actions of somatostatin on the CNS have been demonstrated following systemic³, cerebroventricular⁴, direct intracerebellar⁵ and microiontophoretic^{6,7} application. Furthermore, somatostatin is concentrated in nerve terminals in various regions of the CNS^{8,9} and can be released in a calcium-dependent manner from slices of rat amygdala and hypothalamus¹⁰. The exact nature of the postsynaptic action of somatostatin on neuronal excitability remains unclear, however, and there have been reports of both excitatory^{5,7} and inhibitory^{6,11} effects. We report here our investigations on the action of somatostatin on cell bodies of hippocampal CA1 and CA2 pyramidal neurones. These neurones lie postsynaptically to high densities of nerve terminals shown by immunohistochemical techniques to contain somatostatin-like material¹². Somatostatin had a potent excitatory effect.

Intracellular recordings were made from pyramidal cells in the CA1 and CA2 regions of rat hippocampus, using an *in vitro* preparation of superfused slices as described previously¹³. Somatostatin was applied close to the recording site by microiontophoresis, direct pressure injection or by local application of small droplets.

Intracellular recordings were obtained from a total of 15 cells in 8 separate preparations. The effect of somatostatin on a

CA1 pyramidal cell is shown in Fig. 1, and similar responses were seen in all of the other cells on which it was tested. Application of somatostatin into the cell body layer, by the passage of a negative current of 196 nA through the peptide solution in an iontophoretic electrode, caused a depolarisation (Fig. 1A) and an increase in excitability (Fig. 1B). Within 10 s the number of action potentials evoked by a depolarising current pulse injected through the recording microelectrode increased from two to six, and the latency to the first action potential on the pulse was reduced from 86 to 60 ms. This increase in excitability was accompanied by a depolarisation of 21 mV but no change in membrane resistance (resting value 4.5 M Ω). When the release of somatostatin was terminated, both the excitability and the membrane potential returned to their original values within 30 s.

The magnitude of the excitation was dependent on the amount of negative current passed through the somatostatin solution, and the threshold for a detectable excitatory effect on the cell depicted in Fig. 1 was -56 nA. This excitation could be mimicked by application of glutamate (+20 nA) from an adjacent barrel of the microiontophoretic pipette (Fig. 1C). However, in contrast to the rapid onset of the response to application of somatostatin or glutamate, the excitation of this cell by acetylcholine was slow, with a latency of 60 s, similar to that described previously¹⁴. The excitatory effects of somatostatin were comparable in latency and magnitude to those of glutamate in four other cells tested in this way, and in all cells the effects were only slightly attenuated when the negative current passed through the solution was compensated by a positive current of similar magnitude passed through an adjacent barrel containing sodium chloride. The excitability of the cells was unaltered during the passage of similar amounts of negative current through the barrel containing sodium chloride

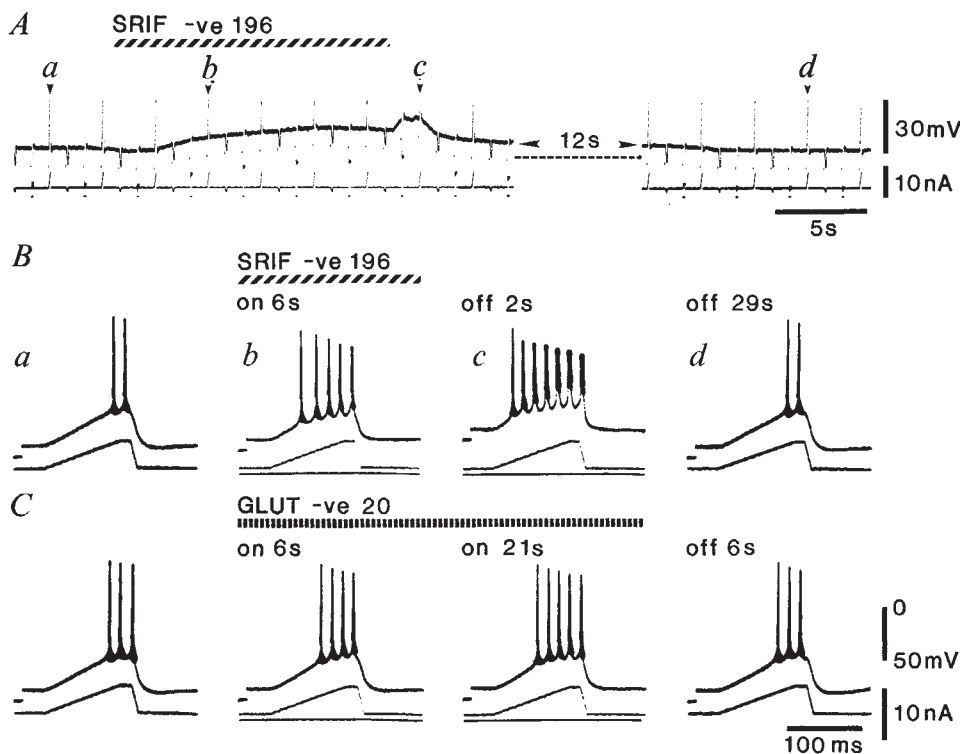


Fig. 1 The effect of somatostatin on a CA1 pyramidal neurone, recorded with an intracellular microelectrode in a rat hippocampal slice preparation. Slices of rat hippocampus, 250 μ m thick, were prepared as before¹³. Recording microelectrodes were filled with 1 M potassium acetate (tip resistance 90–150 M Ω). The centre of a four-barrelled microelectrode was filled with a 3 mM solution of somatostatin (Bachem) in 5 mM sodium acetate (pH 7.0) and the peripheral barrels with 1 M sodium chloride, 1 M sodium acetate, 0.5 M acetylcholine chloride (pH 4.5) or 1 M monosodium L-glutamate (pH 6.7). A backing current of 25 nA and appropriate polarity was applied to each barrel throughout the experiment. In some experiments synthetic somatostatin was used with similar results. Somatostatin was tested only on cells from which stable intracellular recordings of resting membrane potentials of > -60 mV could be obtained. **A**, Somatostatin (SRIF), applied by passage of 196 nA negative current, caused a depolarisation of 21 mV (a resting potential of -75 mV).

Termination of the application resulted in the release of some voltage coupling between the recording and iontophoretic electrodes, which masked an even greater depolarisation. The depolarising pulses and both the evoked and spontaneously occurring action potentials disappeared when photographic conditions suitable for the reproduction of the changes in d.c. level at high gain were selected. **B**, The same record at a faster sweep speed shows the effect of somatostatin on the excitability of this cell at times a–d indicated in **A**. The lower trace shows the depolarising current pulse injected through the recording electrode, adjusted so as to evoke two action potentials in resting conditions.

C, Similar records show the excitation of this cell by L-glutamate.

or sodium acetate, or of larger amounts of positive current through the somatostatin solution.

The excitatory actions of somatostatin could also be demonstrated within as little as 10 s of applying a pressure of 1 to 2 kg cm⁻² to a single peptide-containing pipette (3 mM somatostatin in 5 mM sodium acetate, pH 7; tip diameter 1–2 µm) positioned in the cell body layer, or to the centre somatostatin-containing barrel of the four-barrelled iontophoretic pipette (total tip diameter < 8 µm). Similar excitations could be evoked by application of the same pressure to the glutamate-containing centre barrel of other pipettes (total tip diameter < 10 µm). Other cells were excited by small droplets of somatostatin (3 mM in 5 mM sodium acetate) applied to the taper of the multi-barrelled microelectrode so that they ran down onto the preparation in the immediate vicinity of the recording microelectrode. Droplets of 5 mM sodium acetate alone failed to evoke any change in excitability.

In conclusion, somatostatin applied to the cell bodies of neurones in the pyramidal layer of the CA1 and CA2 regions of the rat hippocampus resulted in a strong excitation which was fast in onset and thus resembled that evoked by glutamate. In contrast, the onset of excitation of these neurones by acetylcholine is considerably delayed, whether the site of application is somatic or dendritic. These results support the hypothesis that somatostatin is a transmitter in the mammalian CNS.

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- Vale, W., Rivier, C., Palkovits, M., Saavedra, J. M. & Brownstein, M. *Endocrinology* **94**, A146 (1974).
- Brownstein, M., Arimura, A., Sato, H., Schally, A. V. & Kizer, J. S. *Endocrinology* **96**, 1456–1461 (1975).
- Plotnikoff, N. P., Kastin, A. J., & Schally, A. V. *Pharmac. Biochem. Behav.* **2**, 693–696 (1974).
- Segal, D. S. & Mandell, A. J. in *The Thyroid Axis Drugs and Behaviour*, (ed. Prange, A. J. Jr) 129–133 (Raven, New York, 1974).
- Rezek, M., Havlicek, V., Hughes, K. R. & Friesen, H. *Neuropharmacology* **15**, 499–504 (1976).
- Renaud, L. P., Martin, J. B. & Brazeau, P. *Nature* **255**, 233–235 (1975).
- Ioffe, S., Havlicek, V., Friesen, H. & Chernik, V. *Fedn Proc.* **36**, 364 (1977).
- Epelbaum, J., Brazeau, P., Tsang, D., Brawer, J. & Martin, J. B. *Brain Res.* **126**, 309–323 (1977).
- Hökfelt, T., Efendic, S., Johansson, O., Luft, R. & Arimura, A. *Brain Res.* **80**, 165–169 (1974).
- Iversen, L. L. *et al.* *Nature* **271**, 679–680 (1978).
- Randic, M. & Miletic, V. in *Iontophoresis and Transmitter Mechanisms in the Mammalian Central Nervous System* (eds Ryall, R. & Kelly, J. S.) 124–126 (Elsevier, Amsterdam, 1978).
- Petrusz, P., Sar, M., Grossman, G. H. & Kizer, J. S. *Brain Res.* **137**, 181–187 (1977).
- Dingledine, R., Dodd, J. & Kelly, J. S. *J. Physiol., Lond.* **269**, 13P–15P (1977).
- Dingledine, R., Dodd, J. & Kelly, J. S. *J. Physiol., Lond.* **273**, 78P–80P (1977).

CXBK mice deficient in opiate receptors show poor electroacupuncture analgesia

RECENT research has led to the hypothesis that acupuncture produces analgesia through the release of endorphins. In neurophysiological studies on single neurones in lamina 5 of cat spinal cord, electroacupuncture reduced responses to noxious stimuli, and the delays of this effect suggested a hormonal mediator¹. Analgesia due to electroacupuncture on mice² and

acupuncture on humans³ can be prevented by the opiate receptor antagonist naloxone. These studies strongly suggest that endorphins may mediate the observed analgesia. Endorphins, specifically β -endorphin and the enkephalins, are peptides whose abilities to bind to opiate receptors^{4,5} and produce analgesia^{6–8} have been well characterised. The implication of endorphins in electroacupuncture analgesia has rested heavily on the ability of naloxone to block the acupuncture effect. Reliance on this single line of evidence has been criticised⁹, since naloxone may possess unknown activities unrelated to its opiate antagonist role. We show here that mice of the CXBK strain which are deficient in opiate receptors show poor electroacupuncture analgesia. This constitutes a naloxone-independent test of the hypothesis.

Baran *et al.*¹⁰ reported genetic differences in brain opiate receptor concentrations in various inbred strains of mice. One of these lines, designated CXBK, demonstrated a lower receptor density than other strains tested. In our study, this 'receptor deficient' strain was compared to another line which possessed a significantly higher concentration of opiate receptors—C57BL/6By, one of the parent strains from which the inbred CXBK line was originally derived. Electroacupuncture was administered to restrained mice (male, aged 7–11 weeks) by means of fine electrodes inserted at a point analogous to the

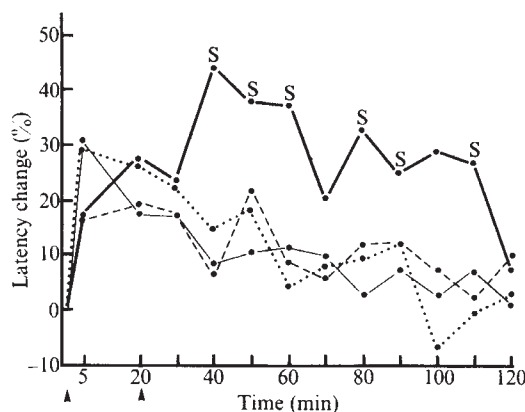


Fig. 1 Analgesia produced by electroacupuncture. Analgesia is expressed as percentage change (from pretreatment control) in latency of hindlimb withdrawal from noxious heat stimulus. Heavy solid line, saline treated C57BL/6By (normal opiate receptor concentration). Light solid line, saline treated CXBK (deficient in opiate receptors). Dashed line, naloxone injected CXBK. Dotted line, naloxone injected C57BL/6By. Electroacupuncture started after zero time and continued for 20 min (arrows). Sampling times showing significant differences between the saline treated groups are designed by 'S'. Each treatment group (each line) represents the average of 15 animals. There was no significant difference in baseline pain thresholds between the two strains (*t* test, two-tailed, *P* > 0.2). For strain C57BL/6By, threshold was 3.17s (s.d. = 0.83 s); for CXBK this was 3.26 s (s.d. 0.66 s).

traditional Chinese acupuncture point, Ho-Ku (L.I.4). This point is located on the forepaw between the first and second digits. Electrical stimulation was given for 20 min in the form of d.c. square pulses of 6–9 V, 1 ms in duration, and at a frequency of 4 Hz. Either naloxone (1 mg per kg body weight) or saline was injected subcutaneously immediately before electroacupuncture and immediately after cessation in a 'blind' experimental design. Pain threshold was determined as the latency for hindlimb withdrawal on exposure to a fixed radiant heat stimulus. The animals were tested before electroacupuncture to establish baseline latencies, and retested after 5 and

20 min of electroacupuncture treatment and at 10-min intervals thereafter for a further 100 min.

Among saline treated animals, the C57BL/6By strain consistently demonstrated a significant analgesia (expressed as a percentage increase in response latency), whereas the opiate receptor deficient CXBK strain showed markedly less analgesia (Fig. 1). Naloxone treated C57BL/6By mice showed a clearly attenuated analgesia in comparison to saline treated C57BL/6By mice. Naloxone treated CXBK mice exhibited a small analgesia similar to the saline-treated CXBK animals. Thus, only C57BL/6By mice showed a marked electroacupuncture analgesia, and it was naloxone reversible.

Genetic variations have previously been reported in the analgesic response of mice to morphine administration¹¹⁻¹⁴. Morphine analgesia in the CXBK strain was shown to be less than that seen in the C57BL/6By strain¹³. It therefore seems plausible that the difference in analgesic response of the two strains we tested is in fact due to an opiate receptor deficiency in the CXBK strain. There is a large body of evidence to implicate an endogenous opioid system in the modulation of sensory information subserving pain. It is well known that stimulation of specific regions of the brain, such as the periaqueductal and periventricular grey matter and raphe nuclei, produces profound analgesia which is partially naloxone reversible^{15,16}. Morphine microinjection into these regions mimics electrical stimulation¹⁷. There also seems to be some correlation between opiate receptors, assayable endorphins, and regions in the brain presumed to be involved in pain sensation. In light of the evidence implicating endorphins in pain modulation, our finding that opiate receptor deficient animals exhibit a decreased electroacupuncture analgesia supports the idea that endorphins mediate these electroacupuncture effects. This approach avoids the criticisms of previous naloxone-dependent experiments, as we have relied not on a pharmacological intervention with its attendant pitfalls, but rather a genetic deficiency in opiate receptors. This same genetic factor in electroacupuncture may be of relevance to clinical findings; presumably, some patients might be genetically more susceptible to acupuncture than others. Moreover, the Chinese reportedly select their patients on the basis of a clinical history of high sensitivity to drugs (including opiates).

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1. Pomeranz, B., Cheng, R. & Law, P. *Expl Neurol.* **54**, 172-178 (1977).
2. Pomeranz, B. & Chiu, D. *Life Sci.* **19**, 1757-1762 (1976).
3. Mayer, D. J., Price, D. D. & Rafii, A. *Brain Res.* **121**, 368-372 (1977).
4. Hughes, J., Smith, T., Morgan, B. & Fothergill, L. *Life Sci.* **16**, 1753-1758 (1975).
5. Waterfield, A. A., Smokcum, R. W. J., Hughes, J., Kosterlitz, H. W. & Henderson, G. *Eur. J. Pharmac.* **43**, 107-116 (1977).
6. Buscher, H. H. *et al. Nature* **261**, 423-425 (1976).
7. Malik, J. B. & Goldstein, J. M. *Life Sci.* **20**, 827-832 (1977).
8. Meglio, M. *et al. Proc. natn. Acad. Sci. U.S.A.* **74**, 774-776 (1977).
9. Hayes, R., Price, D. D. & Dubner, R. *Science* **196**, 600 (1977).
10. Baran, A., Shuster, L., Eleftheriou, B. E. & Bailey, D. W. *Life Sci.* **17**, 633-640 (1975).
11. Brase, D. A., Loh, H. H. & Way, E. L. *J. Pharmac. exp. Ther.* **201**, 368-374 (1977).
12. Eidelberg, E. *et al. Eur. J. Pharmac.* **32**, 329-336 (1975).
13. Shuster, L., Webster, G. W., Yu, G. & Eleftheriou, B. E. *Psychopharmacologia* **42**, 249-254 (1975).
14. Castellano, C. & Oliverio, A. *Psychopharmacologia* **41**, 197-200 (1975).
15. Adams, J. E. *Pain* **2**, 161-166 (1976).
16. Akil, H., Mayer, D. J. & Liebeskind, J. C. *Science* **191**, 961-962 (1976).
17. Yeung, J. C., Yaksh, T. L. & Rudy, T. A. *Pain* **4**, 23-40 (1977).

Transmitter output increases in an identifiable lobster motoneurone with growth of its muscle fibres

IN developing neuromuscular synapses the quantal output of transmitter increases, ensuring sufficient transmitter to produce a propagated action potential in vertebrate muscle fibres¹⁻⁹ and uniform depolarisation of invertebrate muscle fibres^{10,11}. On reaching adulthood further growth in fibre diameter and synaptic output cease in animals with a determinate growth pattern^{12,13}. However, in animals with indeterminate growth pattern, the muscle fibre continues to grow in size. In such animals, in order to maintain equal levels of depolarisation, an increase in transmitter output or changes in the muscle membrane cable properties must occur with growth. We report here that in the lobster *Homarus americanus* which lacks a terminal moult, the level of depolarisation is similar between identified muscle fibres from small and large adult lobsters even though the fibres from the large lobster increases twofold in diameter. This is achieved by an increase in transmitter output in the neuromuscular synapses of large lobsters rather than any compensatory changes in postsynaptic cable properties.

The proximal accessory flexor muscle (PAFM) in the first walking leg of lobsters *H. americanus* was exposed together with its nerve and held at 13°C. The preparation was continually superfused with lobster saline of the following composition: NaCl, 427 mM; KCl, 10 mM; CaCl₂, 16 mM; MgCl₂·6H₂O, 7 mM; glucose, 11 mM. The saline was buffered to pH 7.4 with Tris-HCl (10 mM) and maleic acid (10 mM). The single excitor axon to the muscle was stimulated with brief (<0.1 ms) electrical pulses and the resulting excitatory post-synaptic potentials recorded by intracellular electrodes (e.p.s.ps) or by focally placed extracellular electrodes (e.r.s.ps). Synapses along the length of a fibre in the PAFM are physiologically identical, but differ amongst individual muscle fibres¹⁴. Thus we chose two physiological diverse types of synapse on separate muscle fibres, characterised by the size of their e.p.s.ps and a facilitation index (F_e) which is the ratio of the e.p.s.p amplitude at 10 Hz compared to that at 1 Hz (ref. 15). The two types of synapse were those that released a comparatively small amount of transmitter at low stimulation frequencies but which facilitated considerably at higher frequencies, that is, low-output, high F_e type, and those that released a large amount of transmitter at low stimulation frequencies but did not facilitate markedly at higher frequencies, that is, high-output, low F_e synapse. Furthermore the low-output high F_e type occurred on fibres located adjacent to the principal leg nerve while the high-output, low F_e type were on fibres most distant from the nerve. This regional distribution of physiologically different synapses on a small number (six to nine) of muscle fibre enabled us to examine the same type of fibre if not the same fibre from animal to animal.

Growth of low and high F_e synapses was followed by examining two different size groups—small and large lobsters (Table 1)—in which there was a tenfold difference in weight and a twofold difference in meropodite length. Associated with this growth was a corresponding twofold increase in the diameter and length of the muscle fibres. The chronological difference between our small and large adult lobsters is difficult to determine as lobster growth has only been followed up to 6-7 yr at which time they are sexually mature and weigh 400-500 g. The tenfold difference in weight between our small and large lobsters suggests a difference of at least several years particularly as lobsters do not double their weight at each moult and as the rate of moulting decreases with age.

Table 2 compares the two types of synapse and reveals that the amplitude of the e.p.s.p. and the degree of facilitation

Table 1 Comparison of body parameters and fibre properties of the proximal accessory flexor muscle in small and large lobsters

Size group	Animal weight (g)	Meropodite length (mm)	Fibre length (mm)	Fibre diameter (μm)
Small	307 $\pm$ 114 (54)	3.4 $\pm$ 0.98 (158)	14.3 $\pm$ 0.7 (127)	68.1 $\pm$ 2.7 (111)
Large	3,028 $\pm$ 975 (27)	62.5 $\pm$ 8.4 (83)	30.1 $\pm$ 4.1 (113)	142 $\pm$ 10.6 (62)

Values are means $\pm$ s.e.m.; n given in parentheses.

between low and high F_e synapses is significantly different ($P=0.1\%$ Student's t test) in each size group. However, when the two size groups are compared the level of depolarisation at 1 Hz and the extent of facilitation at 10 Hz produced by low and high F_e synapses remain unchanged ($P>5\%$, Student's t test). In order for the fibres from the large lobster to depolarise to the same extent as their counterparts from small lobsters either transmitter output must increase or appropriate changes must occur in the cable properties of the muscle fibre.

Table 2 Comparison of neuromuscular properties of proximal accessory muscle fibres innervated by two synaptic types in small and large lobsters

Fibre type	Size group	e.p.s.p. (mV)	F_e	n
Low F_e	Small	12.7 $\pm$ 0.9	1.5 $\pm$ 0.07	55
	Large	11.4 $\pm$ 0.7	1.5 $\pm$ 0.1	50
High F_e	Small	0.57 $\pm$ 0.3	4.9 $\pm$ 0.31	62
	Large	0.8 $\pm$ 0.1	4.5 $\pm$ 0.2	63

Values are means $\pm$ s.e.m.; F_e is the ratio of e.p.s.p. amplitude at 10 Hz to that at 1 Hz.

The latter possibility was investigated by comparing the cable properties of fibres from small and large lobsters by using rectangular pulse analysis¹⁶ (Table 3). In each group the membrane electrical properties of input resistance (R_{in}), length constant (λ), time constant (τ_m), sarcoplasmic resistivity (R_i) and specific membrane resistance (R_m) and capacitance (C_m) were similar (Table 3). Thus the cable properties of the muscle fibre cannot account for the observed differences in e.p.s.p. size and F_e in fibres innervated by low and high F_e synapses; these are due to differences in transmitter output. However, when the two size groups are compared clear differences emerge in only R_{in} and λ while the other electrical properties remain unaltered in fibres innervated by each synaptic type. The changes in R_{in} and λ reflect passive responses of the muscle membrane to an increased fibre diameter associated with growth. Furthermore, the change in R_{in} and λ could be predicted from cable theory; that is R_{in} decreased proportionately to the $-3/2$ power of fibre diameter, while λ changed according to the square root of fibre diameter^{16,17}. However, the ratio of λ to fibre length remained equal for comparable muscle fibres between small and large lobsters. If R_{in} is to be considered a measure of post-junctional quantal effectiveness¹⁷, then in order to maintain equal levels of depolarisation for comparable muscle fibres in small and large lobsters, changes in synaptic properties of transmitter output should occur.

This possibility was examined by comparing quantal output (m) which was determined by the failure method at 1 Hz stimulation¹⁸⁻²⁰, between the two size groups (Table 4). There is a significant difference in quantal output between low F_e and high F_e fibres in each size group ($P=0.1\%$ Student's t test). However, between the two size groups the quantal output increased significantly at both high and low F_e synapses in large lobsters as compared to their small counterparts ($P=0.1\%$ Student's t test).

We conclude that the quantal output of transmitter at lobster neuromuscular synapses increases with growth. This ensures that depolarisation of the muscle fibre is comparable during growth. Changes in membrane electrical properties do not influence the level of depolarisation during growth. In fact the change in R_{in} and λ followed the cable theory which is in contrast to development of crayfish opener muscle fibres where only the change in λ was predicted from cable theory¹¹. The decrease in R_{in} with growth of the lobster PAFM indicates the need for an increase in transmitter output during growth. Moreover as the ratio of λ to fibre length remained constant throughout growth, changes in quantal output would be sufficient to ensure uniform depolarisation of the muscle fibre. However, tension studies and an examination of the threshold for E-C coupling in single muscle fibres would verify this point.

Table 4 Comparison of quantal output in proximal accessory muscle fibres innervated by two synaptic types in small and large lobsters

Size group	Low F_e fibre	High F_e fibre
Small	2.3 $\pm$ 0.2 (18)	0.62 $\pm$ 0.2 (12)
Large	3.5 $\pm$ 0.75 (22)	1.4 $\pm$ 0.2 (10)

Values are means $\pm$ s.e.m.; n given in parentheses.

Our results demonstrate that the single excitatory motoneurone which persists for the life of the lobster increases its quantal output of transmitter concomitant with an increase in size of the muscle it innervates. It provides an unusual example of neuronal plasticity as it occurs at a neuromuscular terminal and as it prevails beyond sexual maturity. Furthermore as lobsters have an indeterminate growth pattern their life span may be similar to humans as suggested by the fact that lobsters weighing up to 18 kg have been trapped. In these very large and old animals transmitter output may be predicted to increase as well, thus providing a situation where synaptic plasticity persists during the entire life span of an animal. This is in contrast to animals with determinate growth patterns where synaptic plasticity declines with ageing^{12,13}.

Table 3 Comparison of membrane electrical properties of proximal accessory flexor muscle fibres innervated by two synaptic types in small and large lobsters

Fibre type	Size group	R_{in} (K Ω)	λ (mm)	τ_m (ms)	R_i (Ωcm)	R_m (Ωcm^2)	C_m ($\mu\text{F cm}^{-2}$)	n
Low F_e	Small	644 $\pm$ 26	2.2 $\pm$ 0.15	32.5 $\pm$ 2.2	98.7 $\pm$ 7.9	3276 $\pm$ 315	14.5 $\pm$ 0.9	50
	Large	244 $\pm$ 35	4.2 $\pm$ 0.2	27.8 $\pm$ 4.3	80.2 $\pm$ 11.9	3255 $\pm$ 505	10.3 $\pm$ 2.1	32
High F_e	Small	649 $\pm$ 25	2.1 $\pm$ 0.14	33.2 $\pm$ 2.4	101 $\pm$ 6.6	3457 $\pm$ 217	12.2 $\pm$ 1.0	61
	Large	303 $\pm$ 35	4.4 $\pm$ 0.3	34.6 $\pm$ 3.0	106 $\pm$ 10	4107 $\pm$ 340	12.4 $\pm$ 2.0	30

Values are means $\pm$ s.e.m.

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- Robbins, N. & Yonezawa, T. *J. gen. Physiol.* **58**, 467–481 (1971).
- Shimada, Y. & Fischman, D. R. *Dev. Biol.* **31**, 200–225 (1973).
- Betz, W. J. *J. Physiol., Lond.* **254**, 63–73 (1976).
- Letinsky, M. S. *Dev. Biol.* **40**, 129–153 (1974).
- Bennett, M. R. & Pettigrew, A. G. *J. Physiol., Lond.* **241**, 515–545 (1974).
- Bennett, M. R., McLachlan, E. M. & Taylor, R. S. *J. Physiol., Lond.* **233**, 481–500 (1973).
- Bennett, M. R., Florin, T. & Woog, R. J. *J. Physiol., Lond.* **238**, 79–92 (1974).
- Dennis, M. J. *J. Physiol., Lond.* **244**, 683–702 (1975).
- Dennis, M. J. & Miledi, R. *J. Physiol., Lond.* **239**, 571–594 (1974).
- Govind, C. K., Atwood, H. L. & Lang, F. *Proc. natn. Acad. Sci. U.S.A.* **70**, 822–826 (1973).
- Bittner, G. D. *J. exp. Zool.* **167**, 439–456 (1968).
- Gutmann, E., Hanzlikova, V. & Vyskocil, F. *J. Physiol., Lond.* **216**, 331–343 (1971).
- Vyskocil, F. & Gutmann, E. *Experientia* **29**, 945–946 (1969).
- Frank, E. *J. Physiol., Lond.* **233**, 635–658 (1973).
- Atwood, H. L. & Bittner, G. D. *J. Neurophysiol.* **34**, 157–170 (1971).
- Fatt, P. & Katz, B. *J. Physiol., Lond.* **120**, 171–204 (1953).
- Katz, B. & Thesleff, S. *J. Physiol., Lond.* **137**, 267–278 (1957).
- del Castillo, J. & Katz, B. *J. Physiol., Lond.* **124**, 560–573 (1954).
- Atwood, H. L. & Johnston, H. S. *J. exp. Zool.* **167**, 457–470 (1968).
- Dudel, J. & Kuffler, S. W. *J. Physiol., Lond.* **155**, 514–529 (1961).

Inhibition of active chloride transport by piretanide

THE mechanism of chloride transport in epithelia is still obscure, although it is the dominant process in many tissues, including the loop of Henle^{1,2}, cornea³, gastric mucosa⁴, marine teleost gill⁵ and anterior intestine⁶. In contrast with studies on sodium movements, where ouabain has been a potent tool for defining the role of the sodium pump, the site of action of inhibitors of chloride transport (for example, thiocyanate,

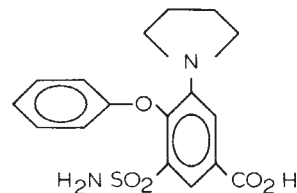


Fig. 1 Structural formula of piretanide.

carbonic anhydrase inhibitors and the diuretics furosemide and bumetanide^{6–9}) remains undefined. We present here data for piretanide¹⁰ (Fig. 1), a new potent inhibitor of chloride transport, which demonstrate that at least one major site of action is at the mucosal entry step in teleost intestine. To do this we have measured short circuit current, conductance, bidirectional chloride fluxes, chloride uptake, intracellular potential and chloride activity following treatment with the drug.

Although fish anterior intestinal mucosa cells are small ($5 \times 40 \mu\text{m}$) microelectrode penetration was routinely successful, enabling us to record steady intracellular potentials for 2–10 min (Fig. 2a). Mean values for membrane potential recorded from sole anterior intestine are given in Table 1. In normal conditions the mean value for the membrane potential (E_m) was -32.5 mV , and the chemical potential (E_{Cl}) recorded by means of a chloride-sensitive electrode (see Table 1) was 27.4 mV (corresponding to an intracellular Cl^- activity of 35.3 mM). Thus, there was a net electrochemical Cl^- potential ($E_m + E_{\text{Cl}}$) of about -5 mV inside the cell, consistent with a higher internal Cl^- activity than would be expected for passive distribution. A somewhat larger non-equilibrium accumulation of intracellular chloride has been reported in a variety of tissues, including gall bladder¹¹ and mammalian intestine^{12–15}. In these tissues, however, where Na^+ -transport can account for the short circuit current, the intracellular Cl^- activities were $15\text{--}50 \text{ mM}$ higher than the values reported here.

When piretanide was added to the mucosal solution there was no immediate change in E_{Cl} , but E_m quickly fell by several

Table 1 Effect of piretanide on electrophysiological parameters of marine teleost intestinal epithelia

	Control	After piretanide (1 mM)	Significance
A Membrane potential (E_m) (mV)	-32.5 ± 1.0 (68:7*)	-33.1 ± 1.3 (68:7*)†	NS
Chemical potential (E_{Cl}) (mV)	27.4 ± 1.0 (58:7*)	32.9 ± 1.1 (58:7*)†	<0.001
Electrochemical potential ($E_m + E_{\text{Cl}}$) (mV)	-5.1 ± 0.4 (58:7*)	0.4 ± 0.7 (58:7*)†	<0.001
Chloride activity (mM)	35.3	29.7†	
B Conductance (G) (mS cm^{-2})	17.8 ± 0.7 (7*)	19.0 ± 0.7 (7*)	NS
Short circuit current (sc) ($\mu\text{A cm}^{-2}$)	-100.1 ± 9.0 (7*)	-39.4 ± 4.2 (7*)	<0.001
Transepithelial potential (E_t) (mV)	-5.69 ± 0.36 (7*)	-2.09 ± 0.22 (7*)	<0.001
Chloride flux (J) ($\mu\text{Eq cm}^{-2} \text{h}^{-1}$)			
J_{ms}	7.11 ± 0.39 (7)	5.31 ± 0.14 (7)	0.01
J_{sm}	2.19 ± 0.34 (7)	2.57 ± 0.36 (7)	NS
J_{net}	4.96 ± 0.45 (7)	2.76 ± 0.37 (7)	0.01
C Chloride uptake ($\text{nmol cm}^{-2} \text{min}^{-1}$)	68.1 ± 3.5 (15:4*)	48.2 ± 2.9 (15:4*)	<0.001

Chloride uptake was measured by a 2-min exposure to Na^{36}Cl and ^3H -inulin medium followed by rapid washing and extraction¹⁶. Bidirectional fluxes using Na^{36}Cl and Na^{77}Br in trace amounts were measured in short-circuit conditions in Ussing chambers. The tissue was equilibrated for 1 h with isotopes and the fluxes measured for 3 h subsequently. Preliminary experiments established the identity of ^{36}Cl and ^{77}Br as tracers for following Cl movements and the stability of the preparation. A and C, sole (*Solea solea*) intestine; B, plaice (*Pleuronectes platessa*) intestine.

Results are ± 1 s.e.m. Numbers in parentheses are no. of observations: *no. of fish.

†Values pooled from a period of 20 min after piretanide administration.

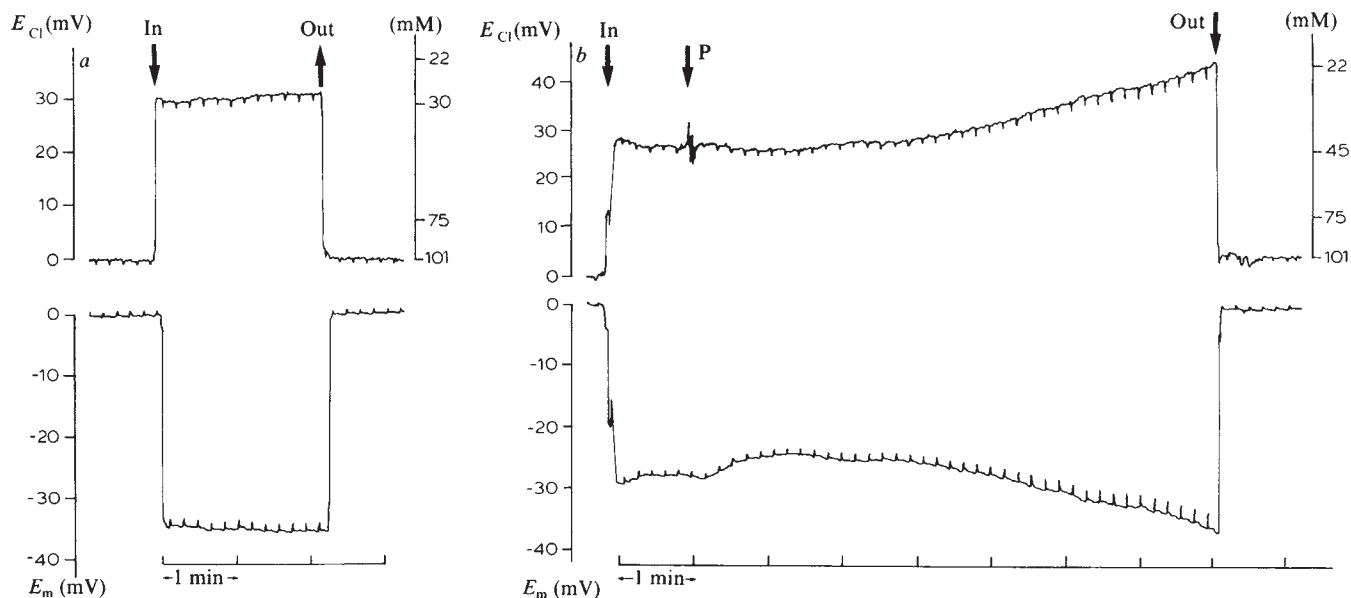


Fig. 2a. The intracellular electrical potential (E_m) and chemical potential (E_{Cl}) of Cl^- were measured with the double-barrelled electrode previously described^{11,17}. The intestine was mounted in a perspex chamber (exposed area 8 mm²) and bathed on both sides in (mM) 130 NaCl, 1.1 MgSO₄, 10 KHCO₃, 2.5 CaCl₂, 25 choline HCO₃, 10 glucose, 2 alanine. The solution was gassed with 95% O₂-5% CO₂, pH about 7.6. The electrode was advanced into the cell from the mucosal side at the arrow marked 'in' and retracted at the arrow marked 'out'. One barrel (the reference barrel) was filled with 2M KCl or 1 M Na₂SO₄ and recorded the electrical potential (relative to a large external electrode), the other was filled with Cl^- -sensitive ion-exchanger (Corning 477315) and recorded a potential closely related to the electrochemical potential of Cl^- . By subtraction and calibration, the chemical potential and the activity (in mM) were obtained. The electrical and chemical potential of the mucosal solution was zero by definition. The Cl^- -electrode gave about 50 mV for a 10-fold change in activity and had a selectivity towards HCO₃⁻ of about 1:30. Any major influence from other ions in these experiments is unlikely because removing Cl^- from the cells by bathing the tissue on both sides for more than 30 min in a saline where Cl^- had been replaced by gluconate caused the intracellularly recorded Cl^- to be smaller than 0.5 mM (concurrently, E_m was increased to about -60 mV). Furthermore, this microelectrode seems to give results in general agreement with Ag/AgCl microelectrodes when used in other intestinal epithelia¹². The tip potential of the reference electrode changed less than 1 mV between isotonic solutions. The impedance of the reference barrel was 50-100 M Ω when filled with 2 M KCl and measured in 2 M KCl; the impedance of the other barrel before being filled with ion-exchanger would have been 10-40 M Ω . **b.** The onset of the effect of piracetamide (added to the mucosal solution) on the intracellularly recorded electrical potential, chemical potential and Cl^- activity was observed 18.8 ± 4.5 (s.e.m. $n = 4$) after application. Some of this delay must be attributed to imperfect stirring in the mucosal compartment. Piracetamide (added at P) initially caused a depolarisation averaging $1.8 \text{ mV} \pm 0.9$ (s.e.m., $n = 4$) (in the example shown, 4 mV), followed by a hyperpolarisation of about 10 mV and a fall in the Cl^- -activity of about 15 mM so as to cause the Cl^- , which initially was accumulated intracellularly against its electrochemical gradient, to attain electrochemical equilibrium across the mucosal membrane. Following a longer exposure to the drug (20 min, see Table 1) the potentials decreased but the chloride distribution remained passive. The same effect of piracetamide was observed whether the reference barrel was filled with Na₂SO₄ or KCl. Further applications of piracetamide did not cause any changes in E_m and E_{Cl} in an already poisoned tissue. An impedance pulse was applied between the reference barrel and the large external electrode every 10 s. The impedance measured between the electrode tip and the large external electrode tended to increase following the administration of piracetamide.

mV, so that in effect the electrochemical potential ($E_m + E_{Cl}$) was reduced. Later (Table 1), both E_m and E_{Cl} increased (Fig. 2b), but after 20 min the membrane was on average slightly but not significantly hyperpolarised, and the electrochemical potential for chloride remained close to zero. The principal effect of the piracetamide was thus to abolish the intracellular accumulation of chloride and render its distribution between the mucosal and the intracellular compartment strictly passive. As seen from Table 1, this was accompanied by a reduction in the ³⁶Cl entry across the mucosal border of about 30%.

The measured short circuit current (Table 1) of $-100 \mu\text{A cm}^{-2}$ (equivalent to $3.7 \mu\text{Eq cm}^{-2} \text{h}^{-1}$) represented 70% of the net Cl^- transport ($5.0 \mu\text{Eq cm}^{-2} \text{h}^{-1}$), with Na⁺ absorption presumably accounting for the difference (for example, see ref. 6). Piracetamide reduced the short circuit current, and inhibited net chloride transport by an equivalent amount (from Table 1, the short circuit current was reduced by $61 \mu\text{A cm}^{-2}$, equivalent to $2.2 \mu\text{Eq cm}^{-2} \text{h}^{-1}$, and the net flux by $2.20 \pm 0.16 \mu\text{Eq cm}^{-2} \text{h}^{-1}$). The effect was rapid, and consistent with the time course of the change in intracellular chloride activity assessed with the microelectrode. Doses as low as 10 μM were effective, but took up to 20 min to achieve the maximal inhibition. We, therefore, used the high dose of 1 mM piracetamide to

allow complete action during the 2-10 min when the microelectrode was inside a cell. No significant change in transepithelial conductance occurred, suggesting that the resistance of the paracellular pathway was not altered by the drug (see Table 1).

Thus, piracetamide is an effective inhibitor of the electrogenic chloride transport in fish anterior intestine. However, even after treatment with the drug a significant net chloride flux and negative short circuit current persisted, suggesting that several components may be involved in chloride transport across this tissue. The results also demonstrate the existence of an electrochemical gradient favouring passive exit of Cl^- from the cell. As Cl^- is transported from mucosa to serosa, chloride transport from the mucosal solution into the cell must invoke an active entry step, probably across the mucosal membrane. Piracetamide inhibited this active entry step, as was also demonstrated by the reduction in Cl^- uptake (Table 1). This inhibition may not necessarily be a direct effect, but could be mediated, for example, by a change in cyclic AMP-protein kinase activity. Therefore, we conclude that electrogenic Cl^- transport in this epithelium is linked to active Cl^- uptake across the mucosal cell membrane, although the Na⁺ pump may still be essential for the net transepithelial transport.

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- Burg, M., Stone, L., Cardinol, J. & Green, N. *Am. J. Physiol.* **225**, 119 (1973).
- Kokko, J. P. *Fedn Proc.* **33**, 25 (1974).
- Zadunaisky, J. A. *Am. J. Physiol.* **211**, 506 (1966).
- Hogben, C. A. M. *Proc. natn. Acad. Sci. U.S.A.* **37**, 393 (1951).
- Maetz, J. & Bornancin, M. *Fortschr. Zool.* **23**, 322 (1975).
- Huang, K. C. & Chan, T. S. T. *Am. J. Physiol.* **220**, 1734 (1971).
- Candia, O. A. *Biochim. biophys. Acta* **298**, 1011 (1973).
- Degnan, K. J., Karnaky, K. J. & Zadunaisky, J. A. *J. Physiol., Lond.* **271**, 155 (1977).
- McGahon, M. C., Yorio, T. & Bentley, P. J. *J. Pharmac. exp. Ther.* **203**, 97 (1977).
- Merkel, W., Bormann, D., Mania, D., Muschaweck, R. & Hropot, M. *Eur. J. med. Chem.* **11**, 399 (1976).
- Zeuthen, T. *J. Membrane Biol.* **39**, 185 (1978).
- Armstrong, W. McD., Wojtkowski, W. & Bixenman, W. R. *Biochem. biophys. Acta* **456**, 165 (1977).
- Frizzell, R. A., Nellans, H. G., Rose, R. C., Markscheid-Kaspi, L. & Schwoltz, S. G. *Am. J. Physiol.* **224**, 328 (1973).
- de Jesus, J., Ellory, J. C. & Smith, M. W. *J. Physiol., Lond.* **244**, 31P-32P (1974).
- Zeuthen, T. & Monge, C. *Phil. Trans. R. Soc. B* **71**, 277 (1975).
- Schultz, S. G., Curran, P. F., Chez, R. A. & Fuisz, R. E. *J. gen. Physiol.* **50**, 1241 (1967).
- Zeuthen, T., Hiam, R. C. & Silver, I. A. in *Ion Selective Microelectrodes* (eds Herman, H. & Herbert, N.) 145-156 (Plenum, London, 1974).

Search for collagen in substrate adhesion site of two murine cell lines

WHEN BALB/c 3T3 cells or their SV40-transformed counterparts are detached from the tissue culture substrate by treatment with the Ca^{2+} -specific chelator EGTA, cellular footpads which mediate the cell-substrate adhesion process are pinched off from the rest of the cell surface and remain as substrate-attached material (SAM)¹⁻³. SAM contains a small percentage of total cellular protein (approximately 1-2% depending on the cell type) and is enriched in glycosaminoglycan-containing proteoglycans, the LETS⁴ glycoprotein (the large, external, transformation-sensitive glycoprotein; also referred to as fibronectin⁵ and very similar to serum-containing cold-insoluble globulin (CIG)⁶), and several proteins associated with the actin-containing microfilaments and 10-nm diameter filaments of these cells^{7,8}. These components seem to be tightly associated with each other as a cell 'surface' complex mediating the adhesive bond³. Evidence is accumulating to indicate that LETS in particular plays an important part in cellular adhesion sites³. LETS (or CIG) has recently been shown to bind to collagen⁹, specifically the $\alpha 1$ chain (H. Kleinman *et al.* personal communication), or to yield immunofluorescence patterns with monospecific antibody similar to those observed for collagen on the surface of some cultured cells¹⁰. Some cell types have been shown to adhere to collagenous matrices after adsorption to the collagen of a serum macromolecule which may be CIG^{11,12}. Therefore, we tried to determine whether the substrate adhesion sites of normal and SV40-transformed BALB/c 3T3 cells contain collagen which might mediate the adhesion process by way of binding of the LETS glycoprotein. Using two independent methods, our studies have demonstrated that very little collagen can be found in these adhesion sites. This indicates that collagen does not mediate the cell-substrate adhesion process of these two murine cell lines and that LETS may bind to some other component in SAM to generate adhesive bonds between the cell surface and the substrate-bound serum receptor molecules³.

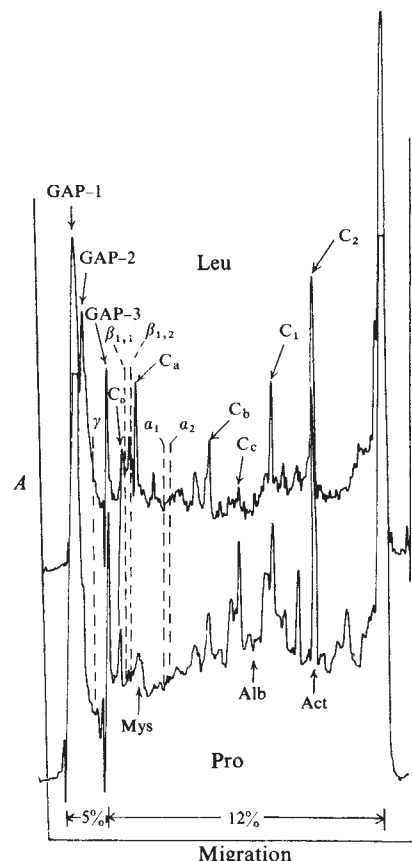


Fig. 1 Electrophoretic comparison of leucine- and proline-radiolabelled SAM. SVT2 cells were grown for 48 h in Eagle's minimal essential medium supplemented with a fourfold concentration of vitamins and amino acids and 10% calf serum in 10-cm plastic tissue culture dishes (Lux). In addition, one set of dishes received $1 \mu\text{Ci ml}^{-1}$ U-L- ^{14}C -proline (240 mCi mmol⁻¹; Amersham/Searle) and a second set $1 \mu\text{Ci ml}^{-1}$ U-L- ^{14}C -leucine (350 mCi mmol⁻¹; Amersham/Searle). At the end of the radiolabelling period, when the cells covered approximately 80% of the substrate surface, the cells were removed by shaking in 0.5 mM EGTA in PBS (5 ml per dish) for 30 min at 37 °C, followed by gentle pipetting⁷. The substrate surface was rinsed three times with PBS. The SAM (which consists essentially of pinched-off footpad adhesion sites and some footprint material^{2,8}) was removed quantitatively by shaking in 0.2% sodium dodecyl sulphate (SDS) plus 2 mM phenylmethylsulphonyl fluoride (to inhibit proteolysis) at 37 °C for 30 min, vacuum dialysed down to a volume 1/50th that of the original, and dialysed against sample buffer (0.2% SDS, 0.07M Tris-sulphate, 15% glycerol, 0.001% bromophenol blue and 1% mercaptoethanol, pH 8.4). 20,000 c.p.m. of ^{14}C -leucine (Leu)- and ^{14}C -proline (Pro)-radiolabelled SAM were electrophoresed in adjacent wells of a 12% Ortec slab SDS-PAGE gel as previously described^{7,8}. The slab gels were stained to indicate where marker myosin (Mys), actin (Act), alb (Alb), and calf skin collagen chains (α_1 , α_2 , β_1 , γ , as indicated by dashed lines) electrophorese. They were then dried and autoradiographed. The autoradiogram was scanned with a Joyce-Loebl densitometer as described previously⁷. Criteria for identifying the following components in SAM have also been described: GAP, glycosaminoglycan-associated protein; C₀, LETS glycoprotein; C_a, myosin; C₁, subunit protein of the 10-nm diameter filaments; C₂, actin^{3,7,8}. Two-dimensional electrophoresis of these extracts has previously shown that all of these protein bands, except C_b, consist of only one protein moiety (L.C., in preparation).

SVT2 cells, an SV40-transformant of BALB/c 3T3 cells which synthesise collagen somewhat more effectively than the 3T3 cells¹³, were grown in medium supplemented with ^{14}C -leucine or ^{14}C -proline during a 48-h period of exponential growth. The SAM from these cells, which contains both foot-

pad (adhesion sites under the cells at the time of the EGTA treatment) and footprint (adhesion sites pinched off the posterior of the cell during natural cell movement across the substrate) adhesive material^{3,8}, was then isolated and analysed on SDS-PAGE gels in reducing conditions as described in Fig. 1, to determine whether there are proline-enriched polypeptides in SAM which electrophorese similarly to the α , β and γ chains of marker collagen. Several SAM proteins have been characterised^{3,7,8}, including the LETS glycoprotein (C_0), and are identified in the figure legend. There are sizable pools of leucine- or proline-radiolabelled LETS in SAM (C_0). There is little, if any, polypeptide material which (a) coelectrophoreses with the α , β and γ chains of calf skin collagen and (b) is proline-enriched. Several other animal collagen samples gave electrophoretic profiles similar to the calf skin material in this SDS-PAGE system. The LETS glycoprotein seems to be somewhat proline-enriched itself relative to the myosin, actin and C_1 (subunit protein of the 10-nm diameter filaments) cytoskeletal proteins. Also, protein C_c seems to be proline-enriched relative to many other proteins. The absence of proline enriched polypeptides which coelectrophorese with carrier collagen chains was also observed when SAM was analysed from cells which had been pulsed for only 2 h; this SAM contains newly formed footpads with a five- to tenfold higher concentration of LETS than long-term radiolabelled SAM⁸. SAM from BALB/c 3T3 cells yielded similar electrophoretic patterns.

An alternative way of determining the collagen content of an extract is based on the ratio of hydroxyproline to proline. The former amino acid comprises as much as 10% of the amino acid

Table 1 Hydroxyproline content in culture fractions

Cell type	Length of radiolabelling* (h)	Culture fraction†	Supplement‡	Hydro/Pro§
SVT2	48	SAM	—	0.0038
SVT2	2	SAM	—	0.0059
SVT2	2	Cell	—	0.0060
SVT2	48	SAM	+	0.0024
SVT2	48	Cell	+	0.0058
SVT2	48	Medium	+	0.018
3T3	48	SAM	+	0.0034
3T3	48	Cell	+	0.0035
3T3	48	Medium	+	0.020

* The indicated cell type was grown in 10-cm plastic tissue culture dishes (Lux) in Eagle's medium supplemented with 3,4-³H-proline (25 Ci mmol⁻¹; New England Nuclear) for 48 h (10 μ Ci ml⁻¹) or 2 h (40 μ Ci ml⁻¹) as indicated during exponential growth of cells. Cultures were fractionated at the end of the radiolabelling period and before the cells had completely covered the plastic tissue culture dish.

† Medium was decanted from dishes and vacuum dialysed at 4 °C, after addition of a few drops of toluene, to a volume 1/50th that of the original. The concentrated medium was then made 0.2% in SDS and dialysed against three changes of 2,000 volumes of 0.2% SDS before hydrolysis. The cell fraction was isolated after washing the cell layer twice with PBS by shaking in 0.5 mM EGTA in PBS (5 ml per dish) for 30 min, followed by gentle pipetting. The suspended cells plus EGTA-solubilised material was frozen-thawed three times and digested with 10 μ g ml⁻¹ of highly purified DNase I (Sigma) at 37 °C for 1 h after making the extract 5 mM in MgCl₂. The extract was vacuum dialysed down to 1/50th volume after addition of SDS to make it 0.2% final concentration. The concentrated extract was dialysed against three changes of 2,000 volumes of 0.2% SDS before hydrolysis. The SAM fraction was isolated as described in Fig. 1.

‡ In cultures indicated with (—), no further additions to the growth medium were made other than radioactive proline. In cultures indicated with (+), the medium was supplemented with 0.5 μ g ml⁻¹ FeSO₄·7H₂O and 25 μ g ml⁻¹ ascorbic acid during the radiolabelling period.

§ Ratio hydroxyproline:proline; conditions for 6M HCl hydrolysis of the protein extracts and analysis for radioactive hydroxyproline and proline on the Bio-Cal 200 amino acid analyser are described in Fig. 2. The ratio represents the amount of radioactivity in the proline peak after correcting for 25% loss of radioactivity as result of hydroxylation of 3,4-³H-proline.

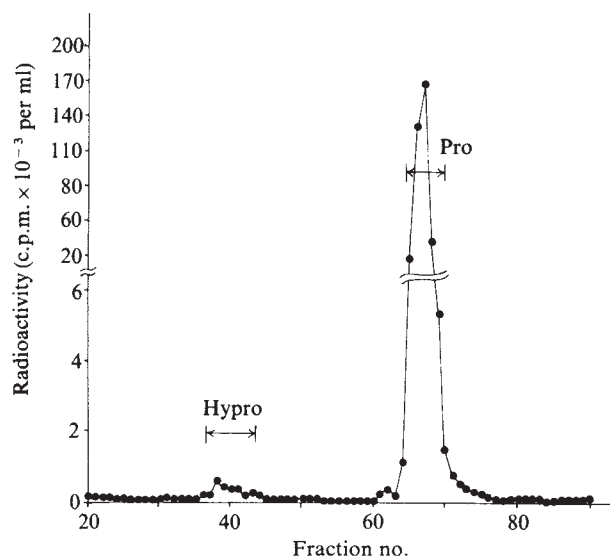


Fig. 2 Hydroxyproline and proline content of SVT2 SAM. Ten 10-cm plastic tissue culture dishes of SVT2 cells were grown for 48 h in MEM $\times$ 4 (10% calf serum) supplemented with 5 μ Ci ml⁻¹ U-L-¹⁴C-proline (specific activity 240 mCi mmol⁻¹). The final density of the cells was 3×10^6 cells per dish and approximately 80% of the surface of the dish was covered with cells. At the end of the radiolabelling period, cells were removed by EGTA treatment and the SAM was removed quantitatively with 0.2% SDS as described in Fig. 1. The SAM (approximately 1×10^6 c.p.m.) was vacuum dialysed to a volume of 0.2 ml, mixed with 3 ml 6M HCl, purged with N₂ gas, evacuated, and hydrolysed at 110 °C for 24 h. The hydrolysate was rotary evaporated to dryness, and the residue was re-dissolved in an aliquot of pH 2.2 starting buffer for analysis in the Bio-Cal 200 amino acid analyser. The amino acids were eluted sequentially with buffers, collected directly from the column into 2-ml fractions, of which 1 ml was used to determine radioactive content in a scintillation counter. Bars indicate regions of elution of marker hydroxyproline (Hypro) and proline (Pro) as determined by ninhydrin analysis.

residues in collagenous proteins and is, therefore, a sensitive indicator of the presence of this protein. ¹⁴C-proline-radiolabelled SAM was obtained from SVT2 cells grown for 48 h in medium not supplemented with Fe²⁺ and ascorbate. When acid hydrolysates of this SAM were chromatographed on the amino acid analyser, the profile shown in Fig. 2 was obtained. A very small amount of radioactivity was observed in the hydroxyproline peak, and the hydroxyproline:proline ratio of the extract was found to be 0.0025. Assuming the hydroxyproline content of collagen to be approximately 10% and the proline content of an average cell protein to be similar to that of actin at 4% (ref. 14), then collagen can be no more than 0.07% of the protein mass of this SVT2 SAM extract. This value is two orders of magnitude less than the LETS glycoprotein content of this extract. In these growth conditions then, collagen seems to be a trace component of SAM.

The hydroxyproline:proline ratio of different cell fractions of BALB/c 3T3 and SVT2 cells has been examined in various growth conditions (Table 1). When 3T3 or SVT2 cells were grown on Fe²⁺- and ascorbate-supplemented medium, the ratio remained quite low in the SAM and cell fractions. Furthermore, there seems to be no enrichment of hydroxyproline-containing material in SAM relative to the EGTA-released cells in conditions in which there is considerable enrichment of the glycosaminoglycan-containing proteoglycans and the LETS glycoprotein^{7,8}. The medium of 3T3 or SVT2 cultures did contain low levels of collagen. When SVT2 cells were pulsed

for 2 h to evaluate the collagen content of newly formed footpads⁸ (Table 1), the hydroxyproline:proline ratio was comparable to the ratios observed in long-term radiolabelled cultures and did not reflect the five- to tenfold elevation of LETS in newly formed footpads. Similarly, there was no enrichment of collagen in the SAM fraction relative to the cell fraction.

These data strongly suggest that collagen is present in trace quantities in the SAM of these two cell lines and probably does not have a significant role in the mechanism of substrate adhesion of these cells. These data would not exclude the presence of a non-hydroxylated procollagen with electrophoretic properties which have not been observed previously using cultured cells. This also does not exclude the possibility that other differentiated cell types or cell lines might use a collagen-dependent process to adhere in an appropriate set of experimental conditions^{11,12}. For example, human skin fibroblasts attaching to tissue culture substrates in serum-free medium deposit a collagenous matrix (with hydroxyproline:proline ratios approaching 0.1) which resists solubilisation with 1 M urea¹⁵. Perhaps the serum coating on substrates provides a receptor molecule, such as CIG¹⁶ (F. Grinnell, personal communication), which interacts with cell surface proteoglycans and/or the LETS glycoprotein, bypassing a requirement for a collagenous matrix. These studies indicate that LETS in the substrate adhesion site of these two murine cell types functions independently of any binding to collagen. What then does it bind to? CIG has been shown to bind to the heparin class of glycosaminoglycans¹⁷. Therefore, two principal candidates seem to be the CIG in the serum coating and/or heparan sulphate recently identified as one of the principal glycosaminoglycans in the SAM of these cells. Consistent with the latter possibility are two recent observations: (1) re-attaching cells deposit SAM containing sizable amounts of heparan sulphate and LETS and very little hyaluronate and chondroitin sulphate (B. Rollins and L.C., in preparation); and (2) enzymatic removal of virtually all the hyaluronate and chondroitin sulphate from SAM using testicular hyaluronidase results in minimal solubilisation of LETS, heparan sulphate and the cytoskeletal proteins (L.C., B. Rollins, J. Buniel and S. Hitri, in preparation). Perhaps the heparan sulphate polysaccharide chains bind to cell surface LETS and substrate-bound serum CIG simultaneously to mediate these adhesive bonds³.

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1. Culp, L. A. *Expt Cell Res.* **92**, 467-477 (1975).
2. Rosen, J. J. & Culp, L. A. *Expt Cell Res.* **107**, 139-149 (1977).
3. Culp, L. A. *Current Topics in Membranes and Transport: Cell Surface Glycoproteins* (ed. Rothstein, A. & Juliano, R.) (Academic, New York, in the press).
4. Hynes, R. O. *Proc. natn. Acad. Sci. U.S.A.* **70**, 3170-3174 (1973).
5. Vaheri, A. & Ruoslahti, E. *J. exp. Med.* **142**, 530-535 (1975).
6. Yamada, K. M., Schlesinger, D. H., Kennedy, D. W. & Pastan, I. *Biochemistry* **16**, 5552-5558 (1977).
7. Culp, L. A. *Biochemistry* **15**, 4094-4104 (1976).
8. Culp, L. A. *J. supramolec. Struct.* **5**, 239-255 (1976).
9. Engvall, E. & Ruoslahti, E. *Int. J. Cancer* **20**, 1-5 (1977).
10. Bornstein, P. & Ash, J. F. *Proc. natn. Acad. Sci. U.S.A.* **74**, 2480-2484 (1977).
11. Klebe, R. J. *Nature* **250**, 248-251 (1974).
12. Pearlstein, E. *Nature* **262**, 497-500 (1976).
13. Culp, L. A. & Black, P. H. *J. Virol.* **9**, 611-620 (1972).
14. Collins, J. H. & Elzinga, M. *J. biol. Chem.* **250**, 5915-5920 (1975).
15. Lembach, K. J., Branson, R. E., Hewgley, P. B. & Cunningham, L. W. *Eur. J. Biochem.* **72**, 379-383 (1977).
16. Grinnell, F. *Expt Cell Res.* **102**, 51-62 (1976).
17. Stathakis, N. E. & Mosesson, M. W. *J. clin. Invest.* **60**, 855-865 (1977).

High viscosity vesicles of yeast separated at pH 4 have surface glycoprotein

MEMBRANE fluidity has an important effect on a wide range of biological phenomena such as transport, protein rotation and diffusion, malignant transformation, enzymatic activities of intrinsic proteins, membrane fusion, and action of anaesthetics. Although experimental modification of membrane fluidity by genetic, environmental, and chemical manipulation has been reported¹, we are not aware of a published method for the convenient isolation of a natural membrane vesicle of high viscosity. We report here on the separation of yeast membrane vesicles into high and low viscosity fractions by pH adjustment.

We used the procedure described by Fuhrmann *et al.*² for the isolation of plasma membrane vesicles capable of galactose transport. A 5,000g pellet from mechanically disrupted cells was resuspended at 1 mg protein ml⁻¹ in ice cold osmotic stabiliser (400 mM KCl, 1 mM MgCl₂, 20 mM triethanolamine). The pH of the vesicle suspension was then lowered from 5.5 to 4.0 by the addition of osmotic stabiliser adjusted to pH 1.2. A dramatic aggregation occurs after pH adjustment yielding an aggregated membrane fraction (A+), which settles in the tube, and a non-aggregated fraction of plasma membrane² vesicles (A-) which remains in suspension. The separation was enhanced by centrifugation at 1,000g for 5 min and A- was collected with a pasteur pipette.

A+ and A- were labelled with a 4×10^{-6} M dispersion of 1,6-diphenyl-1,3,5-hexatriene (DPH) in phosphate buffered saline (PBS) at pH 7.5. Lipid viscosity was determined by DPH fluorescence polarisation^{3,4}. A+ had an average viscosity of 12.5 poise (P) as compared to 3.0 P for A- (Table 1). Viscosity measurements were very reproducible and were not altered after storage of membranes at -70°C for 3 months. Published data⁵ suggested that the high viscosity of A- could result from a high sterol (S) phospholipid (PL) mole ratio. As expected, analysis of lipids extracted from A+ and A- (Table 1) confirmed a much higher S/PL ratio for A- lipids (S/PL = 2.2) in comparison to A+ lipids (S/PL = 0.3). Thus the chemical basis of the decreased fluidity of A-, suggested by the DPH fluorescence polarisation determination, seems to be a relative enrichment for free sterols.

The highest S/PL mole ratio reported for the yeast plasma membrane⁶ is 0.2 in contrast to the value of 2.2 for A-. Thus it seemed that A- isolated by Fuhrmann *et al.*² was a high viscosity domain of the plasma membrane. This possibility was investigated by determining the viscosity of the total plasma membrane isolated by two different methods. The method of Duran *et al.*⁷ utilises concanavalin A (con A) to fortify the plasma membrane of protoplasts prior to hypotonic lysis and membrane purification on a renografin gradient. An unpublished method (H. Bussey, personal communication) uses the enzyme zymolyase⁸ to produce spheroplasts. The plasma membrane adheres to, and is fortified by, a zymolyase-resistant inner layer of glucan. Hypotonic lysis and low speed centrifugation are then used to purify the plasma membrane. Both procedures resulted in a membrane with fourfold lower viscosity (Table 1) than that observed for A-. In addition, a low S/PL mole ratio of 0.4 was observed for lipids extracted from zymolyase ghosts (Table 1). On the basis of these observations we tentatively conclude that A- is enriched for a high viscosity domain of the plasma membrane.

Con A conjugated with ferritin was used for the cytochemical visualisation of saccharide components on A+ and A-. Electron micrographs showed that A- possessed abundant surface carbohydrate in contrast to the virtual absence of con A receptor sites on the outer surface of A+.

Protein from A+ and A- was solubilised and separated on the basis of molecular weight¹⁰ and isoelectric point¹¹ (pI).

SDS-polyacrylamide gels of A+ and A- were stained for protein with Coomassie blue¹²; carbohydrate was identified by staining with periodic acid-Schiff (PAS)¹² and binding¹³ of fluorescein-conjugated con A and ³H-con A. Gels of A- and A+ had similar protein bands but differed by a diffuse band of glycoprotein in A- with an apparent molecular weight of 1.3×10^5 to 2.0×10^5 . (A similar diffuse band of high molecular weight glycoprotein was reported for yeast plasma membrane vesicles isolated by a different procedure¹⁴.) Coomassie blue staining of isoelectric focusing gels also indicated a similar protein composition for A+ and A-. However, PAS staining of gels showed that A- contained a major glycoprotein with an apparent pI of 5.15 that was not detected in A+ (Fig. 1). An additional diffuse major band of A- glycoprotein appeared in the neutral region of the focusing gel (pH > 6.7) as well as several minor components having an apparent pI range between 5.6–6.0 and 6.2–6.4. These components were either not detectable or occurred in very low amounts in electrofocusing profiles of A+. Isoelectric focusing of A-, surface labelled with ¹²⁵I by lactoperoxidase-catalysed iodination, showed that the major surface components were the same glycoproteins (Fig. 1) identified by staining with PAS; major protein bands identified by positive Coomassie blue and negative PAS staining were not surface labelled. These observations demonstrate that A- have surface glycoproteins that are virtually absent in A+. A total carbohydrate determination by the anthrone procedure¹⁶ supports this conclusion since the % carbohydrate (dry wt) was 1.7 for A+ and 7.0 for A-.

The molecular basis for the non-aggregation of A- at pH 4 is not known. However A- have a negative surface charge density from pH 3 to 9 that is quantitatively similar to intact cells². As the negative charge of intact cells results from the ionisation of phosphate¹⁷ present in ester linkages to

Table 1 Viscosity and lipid composition of plasma membrane preparations

Method of isolation	Viscosity (P)*	Total lipid ($\mu\text{M g}^{-1}$)		Molar ratio S/PL
		S*	PL*	
pH aggregation ²				
A-	12.5 ± 0.25	314 ± 22	140 ± 19	2.2
A+	3.0 ± 0.22	142 ± 21	459 ± 22	0.3
Con A stabilisation of protoplasts ⁷	2.3 ± 0.16	—	—	—
Zymolyase ghosts†	2.6 ± 0.15	162 ± 18	466 ± 20	0.4

Viscosity was determined by the measurement (Elscent microviscometer (MV-la)) of the fluorescent polarisation at 25.5°C of DPH embedded in the lipid of membranes^{3,4} (400 μg membrane dry wt). A modified form of the Perrin equation ($r/r_0 = 1 + C(r)(T\tau/\eta)$) was used to calculate viscosity as described^{3,4}; where τ is the excited state lifetime at the absolute temperature T , η is the viscosity of the system, r and r_0 are the measured and limiting fluorescence anisotropies, and $C(r)$ is a parameter that contains volume and shape factors for the fluorophore. τ was estimated to be 8.9 ns by measuring the fluorescence intensity from 0 to 30°C and assuming a r_0 value³ of 11.4 ns. $C(r)$ was assumed to be $8.6 \times 10^5 \text{ P deg}^{-1} \text{ s}^{-1}$ and r_0 was assumed to be 0.362 as reported by Shinitzky and Inbar⁴. Viscosity measurements on protoplasts isolated by con A stabilisation⁷ were made before and after the removal of con A (centrifuged at 100,000g after each of three washes with 0.10 M α -methyl mannoside dissolved in 5 mM Tris-chloride, 2 mM MgSO_4 , pH 7.5). Since the presence of con A had no influence on viscosity the mean of washed and unwashed preparations is given. Membranes were extracted under N_2 with 10 volumes of CH_3OH followed by the addition of 20 volumes of CHCl_3 . The $\text{CH}_3\text{OH}:\text{CHCl}_3$ extract was washed with six volumes of 0.1 M KCl and lipid dry weight was determined on 50 μl samples. Free sterols were converted to trimethylsilyl ethers and quantified by gas-liquid chromatography on a SE-30 column using cholestane as an internal standard⁹. PL was analysed by the determination of inorganic phosphorous⁹.

*Mean $\pm$ s.e.m. from four different preparations.

†Unpublished method developed by Dr Bussey.

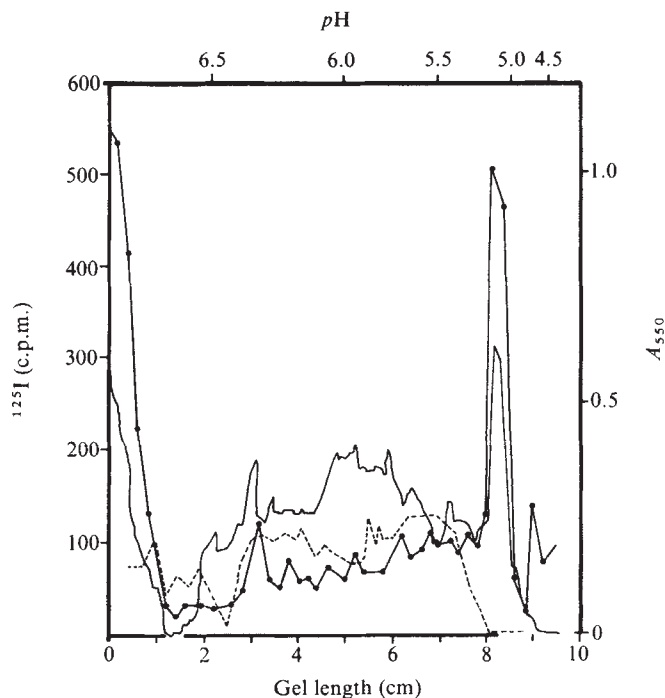


Fig. 1 Polyacrylamide isoelectric focusing gels of A+ and A- membranes. Membrane solubilisation and isoelectric focusing methods were as described by O'Farrell¹¹. Gels were stained for carbohydrate using PAS¹² and scanned at 550 nm. Freshly prepared A- were subjected to lactoperoxidase-catalysed iodination¹⁵ for 20 min at 25°C. The reaction mixture contained 250 μCi ¹²⁵I (carrier free), 8.8 μM H_2O_2 , 20 μl lactoperoxidase (Sigma, 5 mg ml^{-1}), and 3 mg of A- in 1 ml of osmotic stabiliser² at pH 7.0. There was a 10-fold decrease in bound ¹²⁵I in control mixtures lacking H_2O_2 or lactoperoxidase. Gels were loaded with 3×10^5 c.p.m. ¹²⁵I or 150 μg of A+ and A- protein. Radioactive gels were sliced with a Mickle gel slicer into 2 mm sections before counting. ●, ¹²⁵I c.p.m.; broken line, A_{550} for A+; solid line, A_{550} for A-.

mannose¹⁸, the non-aggregation of A- vesicles at pH 4 may be due to the negative charge of ionised phosphate associated with surface phosphomannans.

The surface glycoprotein of A- vesicles does not result *per se* in high viscosity. This is evident as lipids extracted from A- have a high S/PL mole ratio (Table 1), and the liposomes of A- have a greatly increased viscosity compared to liposomes of A+ (unpublished data). It is more probable that a hydrophobic region of the surface glycoprotein spans the lipid bilayer and serves as a focus for a highly viscous lipid domain¹⁹.

In view of the diverse functions associated with membranes and the demonstrated effect of fluidity on membrane functions, it seems probable that domains are present in many membranes^{20,21}. Our observations suggest that surface glycoproteins can be associated with high viscosity domains. Procedures that selectively isolate vesicles with surface glycoprotein may assist in the isolation of such domains.

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- Chapman, D. & Quinn, P. J. *Proc. natn. Acad. Sci. U.S.A.* **73**, 3971–3975 (1976).
- Fuhrmann, G. F., Boehm, C. & Theuvsen, A. P. R. *Biochim. biophys. Acta*, **433**, 583–596 (1976).
- Shinitzky, M. & Barenholz, Y. *J. biol. Chem.* **249**, 2652–2657 (1974).
- Shinitzky, M. & Inbar, M. *J. molec. Biol.* **85**, 603–615 (1974).
- Demel, R. A. & De Kruffy, D. *Biochim. biophys. Acta*, **457**, 109–132 (1976).
- Hossack, J. A., Sharpe, U. J. & Rose, A. H. *J. Bact.* **129**, 1144–1147 (1977).
- Duran, A., Bowers, B. & Cabib, E. *Proc. natn. Acad. Sci. U.S.A.* **72**, 3952–3955 (1975).
- Kitamura, K., Kaneko, T. & Yamamoto, Y. *J. gen. appl. Microbiol.* **20**, 323–344 (1977).
- Van Hoeven, R. P. & Emmelot, P. *J. Membrane Biol.* **9**, 105–126 (1972).
- Ames, G. F. *J. biol. Chem.* **249**, 634–644 (1974).
- O'Farrell, P. H. *J. biol. Chem.* **240**, 4007–4021 (1975).
- Fairbanks, G., Steck, T. L. & Wallach, D. F. H. *Biochemistry* **10**, 2606–2617 (1971).
- Olden, K. & Yamada, K. M. *Analyt. Biochem.* **78**, 483–490 (1977).
- Wehrli, E., Boehm, C. & Fuhrmann, G. F. *J. Bact.* **124**, 1594–1596 (1975).
- Morrison, M. *Meth. Enzym.* **32**, 103–109 (1974).
- Dische, Z. *Meth. Carbohydrate Chem.* **1**, 478 (1962).
- Eddy, A. A. & Rudin, S. D. *Proc. Soc. B* **148**, 419–432 (1968).
- Stewart, T. S. & Ballou, C. E. *Biochemistry* **7**, 1855–1863 (1968).
- Segrest, J. P. & Jackson, R. L. in *Membrane Proteins and Their Interactions With Lipids* (ed. Capaldi, R. A.) 21–46 (Marcel Dekker, New York, 1977).
- Lenaz, G., Sechi, A. M., Parenti-Castelli, G., Landi, L. & Bertoli, E. *Biochem. biophys. Res. Commun.* **49**, 536–542 (1972).
- Mavis, R. D. & Vagelos, P. R. *J. biol. Chem.* **247**, 652–659 (1972).

Interferon action may be mediated by activation of a nuclease by pppA2'p5'A2'p5'A

EXPOSURE of cells to the antiviral agent interferon inhibits virus replication¹. In interferon-treated cells the adsorption, penetration and uncoating of infecting viruses is not affected, but the synthesis of virus-specific mRNA and viral proteins is inhibited^{2,3}. We have studied a mechanism for degradation of mRNA which may be activated in interferon-treated cells by the presence of double-stranded RNA (dsRNA) of viral origin. Lengyel *et al.* have reported that an endonuclease present in extracts of cells treated with interferon is activated by dsRNA and ATP^{4–6}. The endonuclease degrades mRNA by a process which can be divided into two phases: an activation phase requiring the presence of both ATP and dsRNA but not of mRNA, and an endonucleolytic phase, which does not require either ATP or dsRNA⁶. Kerr *et al.* have at the same time reported that protein synthesis is inhibited in extracts of cells treated with interferon after incubation with dsRNA and ATP⁷. In these incubation conditions a low molecular weight inhibitor is formed from ATP^{8,9}. The mechanism of action of this inhibitor, the structure of which has recently been determined as pppA2'p5'A2'p5'A (pppApApA)¹⁰, is not known. Our results indicate that this trinucleotide mediates the activation of a nuclease which degrades mRNA and may therefore inhibit protein synthesis in this way.

The trinucleotide pppApApA was synthesised according to Kerr *et al.*⁹ by first adsorbing the enzyme which catalyses its synthesis from ATP on to a small column of poly I · poly C conjugated to agarose and by then incubating the column-bound enzyme with 1 mM ATP for 17 h at 30 °C. When post-ribosomal supernatant from interferon-treated cells was used for the preparation of column-bound enzyme, the yield of pppApApA was at least 10-fold higher than with post-ribosomal supernatant from control cells (data not shown). This was in agreement with the finding of Kerr *et al.* that extracts from interferon-treated cells are more active in the synthesis of pppApApA than extracts from control cells⁸.

Labelled vesicular stomatitis virus (VSV) mRNA, obtained from infected cells as described previously¹¹, was incubated with extracts from control and interferon-treated cells with or without added pppApApA (Fig. 1). Some degradation of mRNA was observed after incubation in the absence of pppApApA in both cell extracts. The products of digestion

sedimented at the top of the gradient as trichloroacetic acid-soluble material (data not shown), suggesting that an exonuclease activity in the extracts degraded mRNA into mononucleotides. With added pppApApA, mRNA was further degraded in both cell extracts. The degradation was the result of the combined action of exonuclease(s), as the peak at the top of the gradients increased; and possibly of an endonuclease, as the degraded mRNA sedimented in a broad peak between the top of the gradient and undegraded mRNA. A 3'-exonuclease present in HeLa cells has been shown to degrade mRNA and poly A to mononucleotides in a processive way (that is, each RNA molecule attacked is completely digested)¹². A 5'-exonuclease in eukaryotic cells degrades mRNA lacking m⁷G at the 5' terminus and could presumably degrade mRNA fragments^{13,14}. An endonuclease activated by dsRNA and ATP has also been reported by Lengyel *et al.* in an extract from interferon-treated HeLa cells¹⁵. The relevant observation made here is that pppApApA activates a nuclease.

Degradation of mRNA is correlated with the amount of pppApApA added to the incubations (Fig. 2). The trinucleotide activates the nuclease at concentrations as low as 5 nM. With higher amounts of pppApApA, the nuclease activity increases proportionally to the log₁₀ of the trinucleotide concentration. The activity and pppApApA concentration dependence of the nuclease is similar in extracts from control and interferon-treated cells (Fig. 2). In this and the following experiments, degradation of mRNA was measured using an analytical method described previously¹⁶, based on the retention of poly A-containing RNA on oligo dT-cellulose columns. This method does not distinguish between the loss of poly A-containing RNA due to endonucleolytic breaks and that due to exonucleolytic activity.

The time course of mRNA degradation with or without added pppApApA is shown in Fig. 3. There is a progressive decrease in the loss of poly A-containing RNA with time,

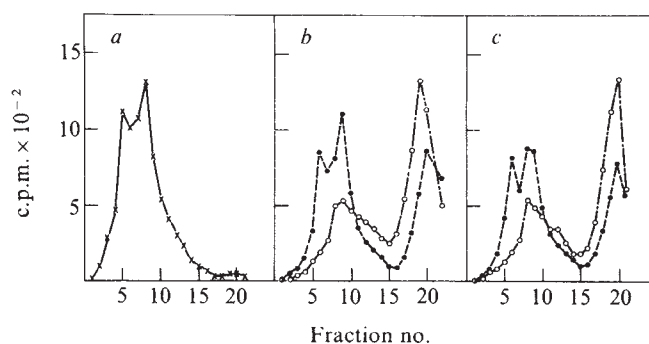


Fig. 1 Activation of nuclease by pppApApA. HeLa cell extracts prepared from control or interferon-treated cells were incubated with ³H-uridine-labelled VSV mRNA. Each incubation contained in a final volume of 55 µl: 30 µl cell extract, 120 mM K(OAc), 1.5 mM Mg(OAc)₂, 10,000 c.p.m. of VSV mRNA, and 280 nM pppApApA where indicated. The cell extracts were prepared as described previously²⁰ from control HeLa cells or from cells treated for 17 h with 100 units ml⁻¹ human fibroblast interferon, obtained from the NCI Interferon Working Group. pppApApA was prepared as described by Hovanessian *et al.*⁹, and VSV mRNA was prepared as described previously¹¹. One incubation was kept at 0 °C, whereas the others were incubated 60 min at 30 °C. The samples were diluted with 0.2 ml 0.1 M NaCl, 1 mM EDTA, 0.5% Na dodecyl sulphate and 10 mM Tris-HCl, pH 7.5, and applied to 5–20% sucrose gradients prepared in the same buffer. The gradients were centrifuged for 17 h at 31,000 r.p.m. in an SW-41 Beckman rotor. Gradient fractions were counted directly with Scintiverse (Fisher). *a*, Unincubated control; *b*, incubations with extract of control cells; *c*, incubations with extract of interferon-treated cells. ●, No pppApApA added; ○, 280 nM pppApApA added.

presumably because the probability that a segment of RNA attached to poly A is cleaved by endonuclease decreases as the segment becomes smaller. When this decreasing probability of cleavage is taken into account in a mathematical analysis of the kinetics of degradation of RNA, a linear rate of RNA degradation with time is observed; this analysis, which is based on the more general treatment of long-chain polymer degradation by Carlesby¹⁷, will be published elsewhere.

Ratner *et al.*¹⁸ established that the dsRNA-activated endonuclease from interferon-treated Ehrlich ascites cells cleaves different RNAs at widely different rates. However, the pppApApA-activated nuclease shows no specificity for either cellular or viral mRNA, nor for free compared with polysome-bound mRNA (Table 1). HeLa cell poly A-containing RNA, either free or polysome-bound, is degraded to approximately the same extent as VSV mRNA. Polysome-bound and free mRNAs are also degraded to approximately the same extent. Some important controls are shown in Table 1: (1) pppApApA does not cause degradation of mRNA in the absence of cell extract; (2) omission of ATP from the incubation with the enzyme bound to poly I · poly C-agarose prevents the formation of the nuclease activator; (3) the presence of ATP is not

required for the activation of the nuclease by pppApApA, as removal of ATP by gel filtration of the cell extract does not lead to a loss of nuclease activity. Moreover, when the non-hydrolysable ATP analogue β , γ -methyleneadenosine-5'-triphosphate (ADPCP) is added to a gel-filtered extract, approximately the same nuclease activity is observed as in the presence of ATP; (4) the nuclease degrades mRNA in the presence of inhibitors of protein synthesis like sparsomycin. In cell extracts incubated with sparsomycin, addition of pppApApA leads to polysome breakdown, whereas in control incubations the polysome pattern remains unchanged after incubation for 1 h (data not shown). Table 1 shows results of experiments designed to measure the degradation of free mRNA, both poly A-containing RNA and total acid-precipitable RNA. In incubations without added pppApApA the degradation of RNA measured in these two ways is approximately the same, suggesting that the RNA is degraded to nucleotides by an exonuclease. However, with added pppApApA the loss of poly A-containing RNA exceeds that of acid-precipitable RNA. This result and the degradation of mRNA bound to polysomes strongly suggest that the enzyme involved has endonucleolytic activity.

Table 1 Effect of pppApApA on nuclease activity of HeLa cell extract

Experiment	Cell extract	Additions or deletions	mRNA	$\frac{\text{c.p.m.}_t}{\text{c.p.m.}_0}$ poly A-RNA	Total RNA	Significance of the experiment
1	None	pppApApA	HeLa	0.90	0.87	Cellular mRNA degraded and cell extract required for degradation
	Control	—	HeLa	0.80	0.82	
	Control	pppApApA	HeLa	0.40	0.66	
	Interferon	—	HeLa	0.87	0.84	
	Interferon	pppApApA	HeLa	0.37	0.61	
2	Control	—	HeLa-polysomal	0.87		Cellular mRNA bound to polysomes degraded
	Control	pppApApA	HeLa-polysomal	0.37		
	Interferon	—	HeLa-polysomal	0.92		
	Interferon	pppApApA	HeLa-polysomal	0.41		
3	Control	—	VSV	0.84	0.79	ATP required for synthesis of nuclease activator
	Control	—ATP in agarose poly I · poly C column	VSV	0.79	0.75	
4	Control-S	—	VSV	0.79	0.81	ATP not required for nuclease activity
	Control-S	pppApApA	VSV	0.60	0.76	
	Control-S	pppApApA + 1 mM ATP	VSV	0.53	0.78	
	Control-S	pppApApA + 1 mM ADPCP	VSV	0.56	0.84	
5	Control	—	VSV/VSV-polysomal	0.90/0.89		Free and bound mRNA degraded at similar rates even when the concentration of pppApApA is decreased 100-fold
	Control	pppApApA	VSV/VSV-polysomal	0.45/0.41		
	Control	1/100 pppApApA	VSV/VSV-polysomal	0.58/0.54		

All the incubations were assembled as described in Fig. 1. The cell extracts were: control, from control cells; interferon, from cells treated for 17 h with 100 U ml^{-1} human fibroblast interferon; control-S, an extract from control cells chromatographed on Sephadex G-25 as described previously²⁰. More than 97% of the ATP was removed from this cell extract by gel filtration (data not shown). The concentration of pppApApA in the incubations was $2.8 \mu\text{M}$, except for the last incubation of experiment 5 in which 28 nM pppApApA was used. In experiments 2 and 5 sparsomycin was added to all the incubations to 0.1 mM final concentration. For experiment 3 ATP was omitted from the incubation with the enzyme bound to poly I · poly C-agarose and an aliquot of the column fraction corresponding to the pppApApA elution peak was used. The mRNAs used in the different experiments were all labelled by incubating with ^3H -uridine control cells and VSV-infected cells as described previously^{11,16}. Control cells were incubated for 3 h in the presence of $0.04 \mu\text{g ml}^{-1}$ actinomycin D. VSV-infected cells were incubated for 4 h after virus infection in the presence of $4 \mu\text{g ml}^{-1}$ actinomycin D. Cell extracts were prepared as described previously²⁰ and polysomes isolated by centrifugation of the cell extracts at $150,000g$ for 90 min. The polysomes were resuspended in $30 \text{ mM K}(\text{OAc})$, $1.5 \text{ mM Mg}(\text{OAc})_2$, 1 mM dithiothreitol, 20 mM HEPES-KOH, $\text{pH } 7.4$, to a concentration of 8 mg ml^{-1} . HeLa cell poly A-containing RNA and VSV mRNA were isolated from aliquots of polysomes by chromatography on oligo dT-cellulose²¹. Approximately $5,000 \text{ c.p.m.}$ of RNA were used for incubation with free mRNA. An aliquot of polysomes containing approximately $5,000 \text{ c.p.m.}$ of labelled poly A-containing RNA was added to incubations with polysome-bound mRNA. In experiment 5 free and polysome-bound VSV mRNA were added together; after the incubation the samples were diluted with 1 ml of buffer used to resuspend polysomes and centrifuged 90 min at $150,000g$ to separate free mRNA in the supernatant from mRNA bound to polysomes. Each fraction was then separately analysed. Control experiments showed that in the conditions of incubation no free VSV mRNA was bound to polysomes, nor was polysome-bound mRNA released, except for a small fraction degraded to acid-soluble material. The poly A-containing RNA, poly A-RNA, was measured by chromatography on oligo dT-cellulose as described previously¹⁶. Total acid-precipitable RNA was measured by addition of 5% trichloroacetic acid. The ratio of the radioactivity of samples incubated for 30 min to that of control samples not incubated is shown. The last column summarises the significance of the different experiments.

We report here three main observations: that the inhibitor of protein synthesis pppApApA can activate a nuclease; that this nuclease shows approximately equal activity in extracts of control and interferon-treated cells; and that the activated nuclease shows no mRNA specificity and degrades equally well free and polysome-bound mRNA.

The pppApApA-dependent activation of nuclease may represent an important mechanism for the degradation of viral RNA in infected cells. Despite the lack of substrate specificity of the nuclease in cell extracts (that is, cellular and viral mRNAs are degraded at an almost identical rate), preferential degradation of viral RNA may occur *in vivo* by the following mechanism. In interferon-treated cells there is a marked increase in the activity of the enzyme synthesising pppApApA. As the enzyme can bind to and is activated by double-stranded RNA, it could bind to the replicative complexes of RNA viruses and catalyse the synthesis of pppApApA at the sites of viral RNA replication. The trinucleotide would activate the nuclease locally and cause preferential degradation of neighbouring RNA molecules, particularly of viral RNA. The trinucleotide may either be destroyed concomitantly with breakdown of mRNA or be relatively short lived. Incubation of labelled pppApApA with HeLa cell sap results in its conversion into smaller adenine containing derivatives (unpublished observations).

Support for the model proposed here comes from an observation of Galster and Lengyel¹⁹. Subviral particles isolated from interferon-treated cells infected with reovirus synthesise short mRNA molecules, whereas subviral particles isolated from control infected cells synthesise full size mRNA. These experiments suggest that a nuclease is associated with the subviral particles isolated from the interferon-treated cells, and raise the possibility that the pppApApA synthetase may also be associated with these particles. Other explanations for these observations are equally possible and experiments are underway to ascertain whether subviral particles obtained from interferon-treated cells have pppApApA synthetase activity.

The model proposed for the degradation of viral RNA in interferon-treated cells is based on a three-phase process:

Fig. 2 Dependency of VSV mRNA degradation on the concentration of pppApApA added. The incubations with extracts from control cells (×) or from cells treated with interferon (●) were assembled as described in Fig. 1. All the samples were incubated for 30 min and the poly A-containing RNA determined by oligo dT-cellulose chromatography¹⁶. Abscissa shows nM concentration of pppApApA present in the incubations; ordinate shows amount of poly(A)-containing RNA remaining after incubation with added pppApApA relative to incubations without added trinucleotide.

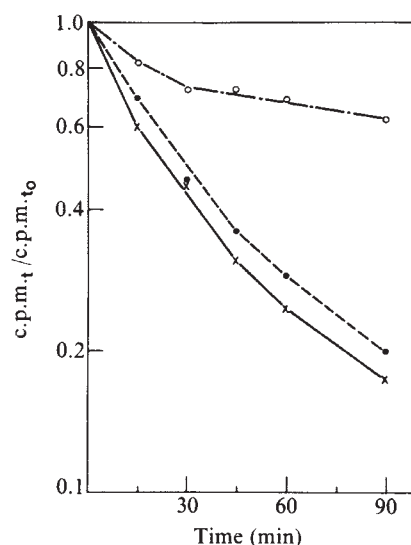
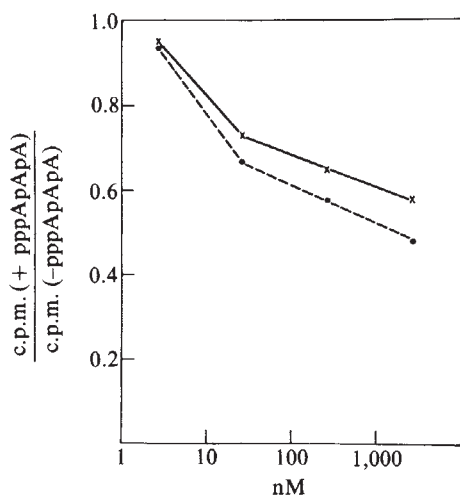


Fig. 3 Time course of VSV mRNA degradation with added pppApApA. The incubations with extracts from control cells or from interferon-treated cells were assembled as described in Fig. 1. Aliquots of the incubations were taken at the times indicated for measurement of poly(A)-containing RNA¹⁶. The amount of poly(A)-containing RNA remaining after incubation with pppApApA relative to that present in a control sample kept at 0°C is shown by the ordinate. ○, Incubation with no added pppApApA and cell extract from control cells (an identical pattern was obtained with extract from interferon-treated cells); ×, 2.8 μM pppApApA added to extracts from control cells; ●, 2.8 μM pppApApA added to extract from interferon-treated cells.

exposure of cells to interferon causes an increase in the amount of active pppApApA synthetase; this enzyme binds to and is activated by dsRNA, with resulting synthesis of pppApApA; and this trinucleotide activates a nuclease which preferentially degrades viral RNA. It remains to be established whether the levels of pppApApA synthetase found in control cells have any physiological role in RNA catabolism and whether this enzyme and the nuclease are part of a pathway for the degradation of RNA which is enhanced on exposure of cells to interferon.

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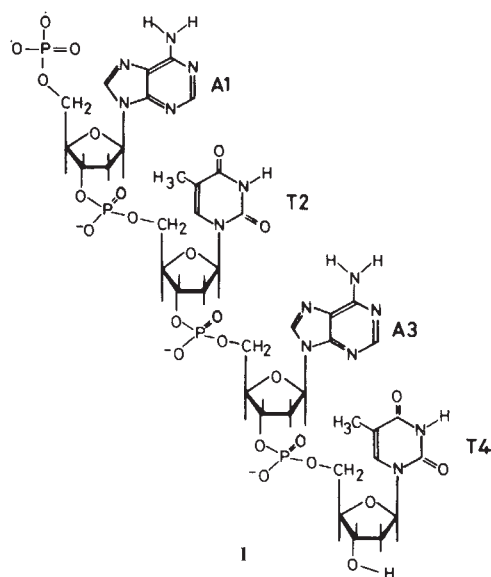
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1. Finder, N. B. (ed.) *Interferon and Interferon Inducers* (2nd edn) (North-Holland, Amsterdam, 1973).
2. Friedman, R. M. *Bact. Rev.* **41**, 543-567 (1977).
3. Metz, D. H. *Cell* **6**, 429-439 (1975).
4. Brown, G. E. *et al. Biochem. biophys. Res. Commun.* **69**, 114-122 (1976).
5. Lebleu, B., Sen, G. C., Shaila, S., Caver, B. & Lengyel, P. *Proc. natn. Acad. Sci. U.S.A.* **73**, 3107-3111 (1976).
6. Sen, G. C., Lebleu, B., Brown, G. E., Kawanita, M., Slattery, E. & Lengyel, P. *Nature* **264**, 370-373 (1976).
7. Kerr, I. M., Brown, R. E. & Ball, L. A. *Nature* **250**, 57-59 (1974).
8. Roberts, W. K., Hovanessian, A., Brown, R. E., Clements, M. J. & Kerr, I. M. *Nature* **264**, 477-480 (1976).
9. Hovanessian, A. G., Brown, R. E. & Kerr, I. M. *Nature* **268**, 537-540 (1977).
10. Kerr, I. M. & Brown, R. E. *Proc. natn. Acad. Sci. U.S.A.* **75**, 256-260 (1978).
11. Shafritz, D. A. *et al. Nature* **261**, 291-294 (1976).
12. Kwan, C. N. *Biochim. biophys. Acta* **479**, 322-331 (1977).
13. Furuchi, Y., LaFiandra, A. & Shatkin, A. J. *Nature* **266**, 235-239 (1977).
14. Shimotohno, K., Kodama, Y., Hashimoto, J. & Miura, K. *Proc. natn. Acad. Sci. U.S.A.* **74**, 2734-2738 (1978).

15. Shaila, S., Lebleu, B., Brown, G. E., Sen, G. C. & Lengyel, P. *J. gen. Virol.* **37**, 535–546 (1977).
16. Hickey, E. D., Weber, L. A. & Baglioni, C. *Biochem. biophys. Res. Commun.* **80**, 377–383 (1978).
17. Carlesby, A. *Proc. R. Soc. A* **224**, 120 (1954).
18. Ratner, L. *et al. Eur. J. Biochem.* **79**, 565–577 (1977).
19. Calster, R. L. & Lengyel, P. *Nucleic Acids Res.* **3**, 581–598 (1976).
20. Lenz, J. R., Chatterjee, G. E., Maroney, P. A. & Baglioni, C. *Biochemistry* **17**, 80–87 (1978).
21. Hickey, E. D., Weber, L. A. & Baglioni, C. *Proc. natn. Acad. Sci. U.S.A.* **73**, 19–23 (1976).

DNA double helical fragment at atomic resolution

DETAILS of the molecular architecture of double helical ribonucleic acids at atomic resolution have recently become available from single crystal X-ray diffraction studies of dinucleotides composed of complementary bases^{1–3}. No similar studies have been published for deoxynucleotides. We report here the structure of the deoxytetranucleotide d-pApTpApT (5'-P-adenylyl-(3'-5')-thymidylyl-(3'-5')-adenylyl-(3'-5')-thymidine) (I) at a resolution of 1.0 Å. This is the first tetranucleotide whose structure has been elucidated by X-ray diffraction. The work is part of an investigation of protein–nucleic acid interactions using single-crystal studies of small model compounds. d-pApTpApT was chosen as one of the first compounds to be examined because there is evidence that poly(dA–dT) has unusual binding properties and that A.T-rich regions in certain DNAs have specific biological roles^{4–7}. We hope that these investigations will provide information about the influence of specific base pairs and sequences on the fine details of the DNA structures, and thus aid the understanding of the selective recognition of nucleotide sequences of the DNA double helix by proteins.



d-pApTpApT was synthesised in this laboratory and crystallised as the ammonium salt with two molecules of the oligomer and 62 water molecules per NH_4^+ ion in a monoclinic cell; space group $P2_1$. The analysis was carried to a resolution of 1.0 Å. Figure 1 shows a view of the molecule.

d-pApTpApT has a self-complementary base sequence, and could, theoretically, crystallise as a self-paired duplex of four base pairs. In the present structure the two molecules in the unit cell are related by a twofold screw axis which eliminates this possibility. A segment of a right-handed antiparallel

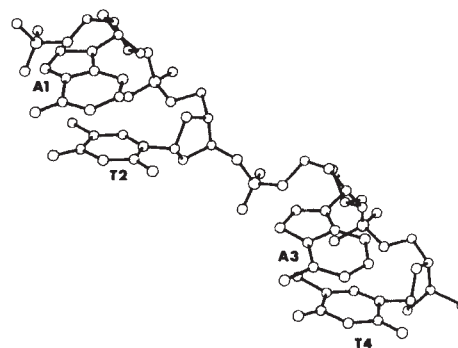


Fig. 1 A molecule of d-pApTpApT viewed perpendicular to *a* and showing the extended backbone conformation and residue labelling.

double helix with two base pairs is, however, formed with complementary hydrogen bonding between adenine and thymine bases related by the 2_1 axis ($A1 \cdot T4^*$, $T2 \cdot A3^*$ where the starred bases are at $1-x, -\frac{1}{2}+y, -z$). The converse pairs ($T4 \cdot A1^*$ and $A3 \cdot T2^*$) have the starred bases at $1-x, \frac{1}{2}+y, -z$; thus, each molecule contributes to two mini-helices, one with each of two other molecules related by *y* translation.

Figure 2 shows a view of the base pairs, held by hydrogen bonds with classical Watson–Crick geometry⁸ (observed for adenine–thymine for the first time in a single-crystal study).

Within each pair the bases are inclined at a dihedral angle of approximately 13° . The mean planes through $A1 \cdot T4^*$ and $T2 \cdot A3^*$ are nearly parallel (3°), with a vertical separation of 3.34 Å. The angular orientation of the base pairs is 31° and the $C1'-C1'$ interstrand separation is 10.2 Å. Such mini-helices have previously been obtained from single-crystal studies of RNA double helical fragments^{1–3} but this is the first description of Watson–Crick base pairs of a DNA fragment at atomic resolution.

A striking feature of the d-pApTpApT molecule and hence of the mini-helix is that the conformation of the deoxyribose is *C3'-endo* when attached to a purine and *C2'-endo* when attached to a pyrimidine base. This is in contrast to double helical fragments of ApU (ref. 1) and GpC (refs 2, 3), where only the *C3'-endo* conformation was found, and the classical interpretation of DNA fibre patterns which were based on monotonic sugar geometry, *C3'-endo* in DNA-A and *C2'-endo* in DNA-B (ref. 9). In the present structure the relative orientation of the base and sugar (χ_{CN}), which is correlated with the sugar conformation¹⁰, also varies between low and high values ($\chi_{CN} = 5^\circ(A1)$, $-9^\circ(A3)$, $69^\circ(T2)$, $75^\circ(T4)$).

Our results suggest that both the sugar pucker and sugar base orientation are 'soft' parameters with low energy barriers between different conformations. It is apparently necessary to consider irregular or periodic variations in these parameters, at least over local regions of double helical structures, depending on the nature of the nucleotide sequence.

An important feature of the structure is the sugar–phosphate backbone (Fig. 1). The conformation within the two halves of the molecule is remarkably similar, with torsion angles ω and ω' about the $P-O5'$ and $P-O3'$ ester bonds in the d(A–T) fragments being close to those allowed for right-handed double helical polynucleotides. The phosphodiester linkage between the two halves, however, is very different, with *trans-gauche* conformation giving rise to an extended backbone similar to that observed in the crystal structure of UpA (refs 11, 12) and pTpT (ref. 13).

The base paired duplex ($A1-T2$)($A3^*-T4^*$) found in the present structure provides a reasonable starting point for generating a model for the dA–dT copolymer.

Model building studies showed that a helical structure could not be generated from the duplex by helical rotation and translation only. It was necessary to alter the conformation of the

phosphodiester bridge in the TA stacked region, changing ω' from 168° to about -100° . The orientation of the base pairs between successive duplexes was also changed from 31° to about 40° . Figure 3 gives a general view of the model. The central portion is a segment of the poly(dA-dT)·poly(dA-dT) model and regions *a* and *b* are the duplex fragments in the positions found in the crystal structure. The model is a qualitative one and a variety of double-helical structures can be built from the ApT fragment by varying the torsion angle ω' (O3'-P) linking adjacent dinucleotides. The relative plausibility of a number of such models has been investigated by energy minimisation calculations (A. Klug, A. Jack, M. A. V., O. K., Z. S. and T. A. Steitz, in preparation).

The most interesting feature of the model is the sugar-phosphate backbone, where the deoxyribose conformation alternates between C2'-endo and C3'-endo and the conformation of the phosphodiester bridge changes significantly between the AT and TA regions. The glycosidic angles for purine and pyrimidine bases are also significantly different (0°

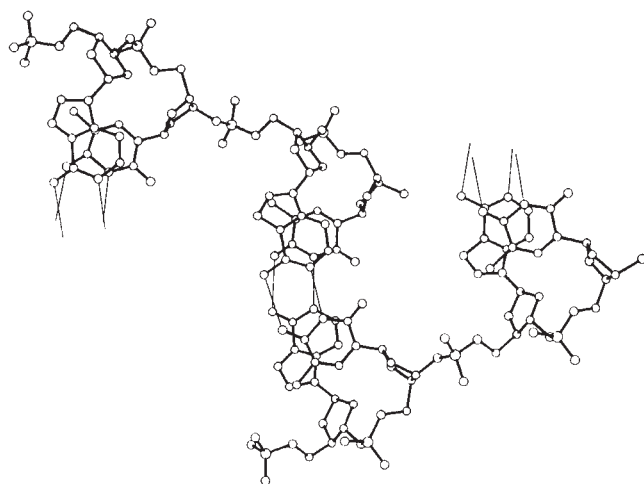


Fig. 2 The mini-helix viewed perpendicular to the base pairs. Hydrogen bonds are indicated by thin lines.

and 70°). The model is characteristic of a deoxyribose structure, as the substitution of a hydroxyl group on C2' of the thymidine moiety of the (dT-dA) fragment is severely hindered sterically. An analogous DNA-RNA hybrid structure is not, therefore, feasible. This would suggest that stretches of poly(dA-dT)·poly(dA-dT) regions in satellite DNAs¹⁴ have a structural rather than a transcriptional role^{9,15}.

There is evidence that AT-rich regions have other specific biological roles, for example, the preferential binding of *lac* repressor to such regions or to poly(dA-dT)·poly(dA-dT) itself⁴.

It has been postulated^{16,17} that these regions may have specific geometric features which can be selectively recognised by proteins involved in the coding process or its gene regulation. The crystal structure suggests a possible way in which such geometric features may arise. A future paper (A. Klug, A. Jack, M. A. V., O. K., Z. S. and T. A. Steitz, in preparation) brings together the evidence of the tetranucleotide crystal structure, the fibre diffraction data and various physico-chemical properties, all of which point to a rather special structure for double-stranded poly(dA-dT). A rationale for this structure is given, which provides an explanation for the binding properties of poly(dA-dT)·poly(dA-dT) to the *lac* repressor of *Escherichia coli*.

Our analysis was based on 2,717 independent, significant structure factors (4,528 diffractometer measurements) and a unit cell of dimensions $a = 21.121(10)$, $b = 21.294(14)$, $c = 8.770(4)$ Å, $\beta = 97.84(4)^\circ$. The structure was solved by a

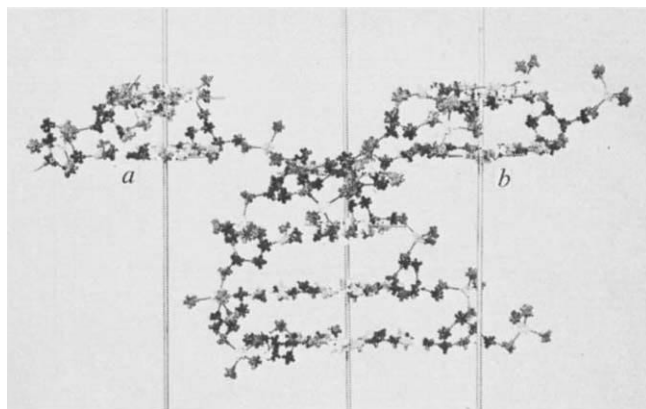


Fig. 3 A photograph of a model of poly(dA-dT) based on the mini-helix: regions *a* and *b* indicate duplex fragments found in the crystal structure.

combination of indirect and direct methods and refined to $R = 15\%$ (isotropic). The water structure is disordered. Full details will be published elsewhere.

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1. Rosenberg, J. M. *et al.* *Nature* **243**, 150-154 (1973).
2. Day, R. O., Seeman, N. C., Rosenberg, J. M. & Rich, A. *Proc. natn. Acad. Sci. U.S.A.* **70**, 849-853 (1973).
3. Hingerty, B. *et al.* *Acta Crystallogr.* **B32**, 2998-3013 (1976).
4. Lin, S. Y. & Riggs, A. D. *Nature* **288**, 1185-1186 (1970).
5. Riggs, A. D., Lin, S. & Wells, R. D. *Proc. natn. Acad. Sci. U.S.A.* **69**, 761-764 (1972).
6. Lin, S. & Riggs, A. D. *Biochem. biophys. Res. Commun.* **45**, 1542-1547 (1971).
7. Wingert, L. & von Hippel, P. H. *Biochim. biophys. Acta* **157**, 114-126 (1968).
8. Watson, J. D. & Crick, F. H. C. *Nature* **171**, 737-738 (1953).
9. Arnott, S., Chandrasekaran, R., Hukins, D. W. L., Smith, P. J. C. & Watts, L. *J. molec. Biol.* **88**, 523-533 (1974).
10. Sundaralingam, M. *Jerusalem Symp. Quantum Chem. Biochem.* **5**, 417-455 (1973).
11. Sussman, J. L., Seeman, N. C., Kim, S. H. & Berman, H. M. *J. molec. Biol.* **66**, 403-421 (1971).
12. Rubin, J., Brennen, T. & Sundaralingam, M. *Biochemistry* **11**, 3112-3228 (1972).
13. Camerman, N., Fawcett, J. K. & Camerman, A. *J. molec. Biol.* **107**, 601-621 (1976).
14. Laskowski, M. *Prog. Nucleic Acid Res. molec. Biol.* **12**, 161-188 (1972).
15. Arnott, S., Fuller, W., Hodgson, A. & Prutton, I. *Nature* **220**, 561-564 (1968).
16. Bram, S. *Nature new Biol.* **232**, 174-176 (1971).
17. Richmond, T. J. & Steitz, T. A. *J. molec. Biol.* **103**, 25-38 (1976).

reviews

Spontaneous generation debate

F. E. G. Cox

The Spontaneous Generation Controversy: From Descartes to Oparin. By John Farley. Pp. 225. (Johns Hopkins University Press: Baltimore and London, 1977.) \$13.50; £10.25.

GREAT scientific controversies never cease to be fascinating, especially if new arguments are produced to challenge widely accepted ideas. The traditional history of the theory of spontaneous generation begins with its acceptance by Aristotle and his contemporaries, its emergence in the Middle Ages, its growth in popularity until the middle of the last century, its gradual relegation to simpler and simpler forms of life, and its final extinction by Pasteur in the 1860s. This is a story of the triumph of the scientific method, of fact built on fact and new discoveries and ideas being incorporated into existing theories. On another level, it is a story with its heroes and villains, wise men and fools, and one in which Pasteur is depicted as the patient searcher after truth whose ideas are ultimately accepted.

John Farley's book is not a story book. It is an extremely well researched historical account of the final years of the spontaneous generation controversy beginning with the period leading up to Pasteur's work and continuing beyond it to the work of Oparin in the 1930s. Farley's thesis is that the controversy did not end with Pasteur but continued for over half a century afterwards and finally died as a result of the ideas of Oparin on the origin of life. Pasteur's contribution is, therefore, relegated from being the final unequivocal statement on this subject to something relevant but not absolutely final. The arguments are supported by a vast selection of documentary evidence, much of it carefully annotated, in an extensive set of notes inserted at the end of the book (mercifully not in footnotes).

There is no doubt that some controversy remained after the 1860s especially in Britain and Germany but, as Farley himself points out, after 1880 any belief in the spontaneous generation of complex organisms had all but disappeared. After the 1880s the debate slowly began to take a new form, not whether known organisms arose *de novo* but whether there were any forms of life that could do so; and even in the 1930s André Gratia was arguing that precellular forms might arise spontaneously. Credit for the theory that life arose slowly as a result of a long history of chemical changes must go to

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Oparin and it is this theory, according to Farley, that finally put an end to the spontaneous generation debate.

This book is fascinating not only for the amount of information it contains but also for two intertwined themes: the influence of religious and philosophical ideas on the state of the debate at any particular time and the relegation of Pasteur's contributions. Both may be questioned. Science historians have long sought to show how scientific ideas have grown out of the social and religious background of their times and have found this dangerous ground to tread. Science obviously emerges as a product of its time but it is practically impossible to say how much the events of a particular period actually influence the scientific achievements of that period, and any opinion tends to be clouded by individual prejudices. We may say now, for example, that smallpox was eradicated in Africa because Africans eventually overthrew the yoke of imperialism or because of the concern of the developed world, or that it was independent of the phases of African history. Perhaps we might be able to express informed opinions now but how much more difficult will this be a century hence? Farley's arguments that the various theories of spontaneous generation were related to religious, political and social changes rather than to the gradual evolution of increasingly rigorous scientific investigation are not convincing.

What of the role of Pasteur? He entered the spontaneous generation debate in 1859 shortly after the death of his much-loved daughter and remained in this field while still continuing his many other activities. This may originally have been a mental exercise, something to shut

out the memory of his daughter. Whatever the reasons for his involvement with spontaneous generation the debate became one between Pasteur and Pouchet; and the prize, which Pasteur won, was convincing the Académie des Sciences that microorganisms abounded in the air and did not arise *de novo* in culture media. Pasteur was a great showman and it is characteristic that he should overstate his case. Perhaps he did but it is those of us who have studied and admired his work who have put him in his present distinguished position, not he himself. It is unlikely that Farley's book will have any effect on Pasteur's reputation.

Did the debate die after Pasteur's work? Farley maintains that it did not but what is ignored is the fact that the later years of the last century and the first years of the present one witnessed an immense expansion of scientific activity, so the spontaneous generation debate occupied a relatively decreasing part of the whole scientific scene. Scientists will debate anything and arguing is often easier than performing definitive experiments. It is not surprising that the debate did continue but few would accept that it, like the pro-Lamarckian argument, was anything more than a gentle murmur against the roar of scientific debate elsewhere.

And the role of Oparin's ideas. Debates die when the debaters stop arguing and go home, and not because of convincing arguments. By the time Oparin had begun to think about the origin of life, modern biology had begun to take form and most scientists had more to do than to participate in sterile arguments. Farley states at one point that the spontaneous generation controversy was almost dead when Pasteur entered the field; he cannot have it both ways and spontaneous generation must have been very dead when Oparin came along.

Perhaps Farley has overstated his case (Pasteur did too) but this makes his book all the more interesting. It is a fascinating book to read and no-one who really wants to know about the beginnings of experimental biology and critical biological thought should avoid reading it. Pasteur's reputation will not be diminished by this book. Oparin's will not be enhanced. But the scientific literature will be enriched by it. □

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Photo: Novosti

Academician Alexander Oparin

Images from Skylab

SKYLAB Explores the Earth. Prepared by NASA Lyndon B. Johnson Space Centre. Pp. 517. (NASA.) £29.50.

SKYLAB represents to space image users and researchers alike, a collection of over 35,000 frames of the highest quality colour, stereoscopic, photographic images yet acquired from a space platform. The actual magazines of film were returned to Earth for processing and development, resulting in the greatest degree of spatial resolution (55'–100') yet obtained on images acquired in space. All other imaging satellite sensing systems rely on the transmission of data by electronic means from the space vehicle to Earth, where images are regenerated with an accompanying degradation of resolution. When I saw the title of this publication from NASA, I had therefore expected a comprehensive selection of high resolution, stereoscopic, photographic images depicting Earth terrain, and oceanic and meteorological patterns and conditions. This expectation was unfulfilled, for in effect, this particular publication deals only with a sampling of some 2,000 frames of hand-held colour imagery (35 and 70mm).

So much for what this publication is not! What it is then is a series of some 17 separate research studies by US Government researchers, university researchers, and private contractors, dealing with projects involved with the physical, biological, cultural, and meteorological aspects of the Earth's environment. Four chapters deal with geological features (tectonics, volcanism, impact structures and physiography); two with various aspects of desertification processes; four with meteorological phenomena; three with oceanography including one on sea ice distribution patterns; and one chapter each with snow distribution, cultural patterns, vegetation patterns, and atmospheric pollution. All this is followed by an overall summary, three appendices, a glossary, a section on weather symbolisation, and a photographic index.

The individual subjects and their treatment with respect to the use of Skylab images consist primarily of enlarged colour images with annotations either on the image itself or presented as a line drawing on the facing page. The volume is profusely illustrated in this manner with most of the images being reproduced in colour on high quality paper. It is unfortunate that many of the images and facing-page line drawings are not

of the same scale or do not always cover the same area, making it difficult to transpose the annotations, by eye, across the page to the image.

Many of the authors of the respective chapters point out the value of being able to view these Skylab images stereoscopically, yet only four stereo pairs are presented in the book. Perhaps it is just as well in that each model is incorrectly spaced (3.4" apart versus a normal 2.4") for three-dimensional viewing by the naked eye. Also in the screen printed reproduction of these photographic images, the attempt to use a stereoscope having any degree of magnification at all, only results in a breakdown of the scene into dots or cells of colour. A photographic process of reproduction and publication would have had to have been carried out to include stereoscopic viewing of any value.

There is one main recurring theme

running through the material presented in this publication. Each study concludes that it is extremely valuable to have man and his decision-making capabilities in space utilising equipment of the highest quality to document and record his observations rather than to solely rely upon remotely controlled instrumentation. In this I would concur, especially when dealing with transitory subjects (amply depicted in this volume). However, I also believe that the best photographic system aboard Skylab was the vertically oriented S-190B Earth Terrain Camera system which was designed to achieve the optimum stereoscopic images of the Earth but which is not displayed here. The best of Skylab is yet to come.

Vernon H. Anderson

Vernon H. Anderson is President of the Photographic Interpretation Corporation, Hanover, New Hampshire.

Europe's threatened wildlife

The Shadow of Extinction: Europe's Threatened Wild Mammals. By Jeremy Mallinson. Pp. 224. (Macmillan: London, 1978.) £5.50.

A PRELIMINARY excursion into this book presents the reader with some puzzlement: for whom was it written and, indeed, why was it written at all? The selection of European mammals, which are dealt with in considerable detail, seems to have been somewhat arbitrary: certainly some of them, for example, the blue hare, the badger and the red squirrel, seem to be far from "the shadow of extinction".

On further perusal, some sort of rationale begins to appear. For one thing, some of the species, for example, the Pyrenean desman and the Mediterranean monk seal, are coming near to extinction and it is valuable to have their distribution, characteristics and prospects chronicled. Again, other species, which flourish still over the main part of their range, for example, the ringed seal and the red deer, have small, isolated populations which may quickly vanish and it is just these outliers that may have interesting biological characteristics, as yet unrecorded. Thirdly, details about many of these species are hard to find in the present literature, though one or two works on Palaearctic mammals are nearing publication.

As Dr Bourlière says in his Foreword, Europe is remarkable in that so few actual exterminations of mammal species have occurred within historic

times, but the threat lies in the decline in numbers and the fragmentation of what were widely spread, continuous populations. Here, Mr Mallinson's information is useful for pin-pointing where the dangers lie and what measures of conservation are needed.

It is when one comes to examine the documentation that one realises the weak points of this book. The bibliography is a remarkable hodge-podge, ranging from popular works like the *Sunday Times* series of booklets on British mammals to a few cardinal works such as van den Brink's *Field Guide to the Mammals of Britain and Europe* and Smit and van Wijngaarden's *Threatened Mammals in Europe*. The latter does not contain quite the same selection of species but it has been relied on very heavily, especially for maps and distributions. In fact, some sections, for example, the world distribution of the walrus, are extracted almost verbatim. Few journal papers are cited and, in these, page numbers are not given. Furthermore, in one instance (Godfrey, 1975), Mr Mallinson has thoughtfully provided a title of his own invention, which makes the reference somewhat difficult to trace.

On the whole, for a scrupulous presentation of the subject, I would recommend Smit and van Wijngaarden's report to the Council of Europe's European Committee for the Conservation of Nature and Natural Resources, though this has no pictures.

H. N. Southern

H. N. Southern recently retired as Senior Research Officer of the Animal Ecology Research Group in the Department of Zoology, University of Oxford, UK.

Relaxation methods in electrochemistry

Transient Techniques in Electrochemistry. By D. D. MacDonald. Pp. 329. (Plenum: New York and London, 1978.) \$45.

As relaxation methods are now extensively used to study electrochemical reactions, there is a definite need for a book which comprehensively deals with the subject in depth. The author has attempted to write such a book, and has succeeded admirably.

After an introductory chapter in which the interphase structure and basic electrode and solution processes are reviewed, the experimental and mathematical methods of treatment to be used in later chapters of the book are described. There follow chapters describing potential step perturbation, current step perturbation, potential sweep techniques, and a.c. impedance methods. The final chapter deals with the electrochemistry of some surface processes.

The format adopted throughout is to describe a particular transient technique followed by a brief description of the experimental method. The

basic equations describing the various systems are established and time-dependent system response obtained by Laplace transform methods.

The experimental circuitry is treated only in principle and actual circuit component details are not given. Therefore, the book is, in itself, not quite adequate for the experimental researcher. However, the important and up-to-date references are all there. The discussion of the theoretical background to the experimental techniques is very well done and is one of the book's strong points. Indeed, the book is unique in fully treating the transient responses of electrochemical circuits.

The theoretical descriptions of the electrochemical systems are of uniformly high quality. Moreover, the mathematical analyses of the transient phenomena are all completely generated within the book's covers, and this contributes enormously to its usefulness. The book is lucidly written and excellently produced. Electrochemists will find the book indispensable, as it is likely to be the standard work on the subject for many years.

N. A. Hampson

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Sequencing linear polymers

Polymer Sequence Determination: Carbon-13 NMR Method. By J. C. Randall. Pp.155. (Academic: London and New York, 1977.) \$16.50; £11.70.

THE rapid development of pulsed Fourier transform methods for obtaining carbon-13 nuclear magnetic resonance (NMR) spectra of organic molecules has revolutionised the analysis of polymer structures during the past few years. Whereas proton NMR spectra are complicated by spin-spin interactions between magnetic nuclei, this effect is missing from the carbon-13 spectra, in which all of the information is present in the form of chemical shift differences, each unique carbon atom giving rise to a single sharp signal. In practice subtle variations in the spectra of polymers arise from chain branching, isomerisation of monomer units, inversions in head-to-tail sequences of monomers, and stereochemical sequences in homopolymers, with the complication of monomer sequence distributions in copolymers.

This book provides a brief but comprehensive account of the use of the NMR method for determination of chemical and stereochemical sequences in linear polymers. In the first two chapters the configurational statistics

of a homopolymer chain are described; using polypropylene as example, it is shown how the NMR spectrum can be interpreted in terms of configurational sequences, and the number average sequence lengths computed. Similar assignments for binary copolymers follow and there is an excellent description of the use of statistical models of polymer chains to assist in spectral assignment. Most of the remaining space is occupied by a review of carbon-13 studies of vinyl polymers and copolymers.

The book is written in the language of the polymer scientist, and little knowledge of the NMR phenomenon is expected. There is, however, an excellent short chapter on the experimental requirements for successful application of carbon-13 NMR to polymers. The spectra used to illustrate the text were all obtained by the author in his own laboratory and are of excellent quality. Although this is not a book to be read for pleasure, it is an indispensable reference work for anyone involved in the interpretation of carbon-13 spectra of polymers and can be strongly recommended.

My only regret is that there is no discussion of the analysis of chain branching in polymers, an area in which the NMR method has proved extremely successful.

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(*Series in Analytical Chemistry*, Editors: Dr. R.A. Chalmers and Dr. M. Masson.)

April 1978 362 pages
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Avian breeding cycles

Avian Breeding Cycles. By R. K. Murton and N. J. Westwood. Pp. 594. (Oxford University Press: Oxford, London and New York, 1978.) £20.

ANYONE who is capable of writing an extensive and detailed review of topics ranging from neuroendocrinology to population ecology deserves to be admired. Dr Murton, well known for his work in the fields of ecology and endocrinology, is one of the very few people qualified to attempt such a task. Although this book has a modest title, the contents cover an amazing array of subjects: the anatomy and endocrinology of avian reproductive systems; the energetics of reproduction; breeding cycles; circadian clocks and photoperiodic measurement; sexual selection and mating systems; population regulation; and (briefly) life history theory. Each area is reviewed in some depth, and in those chapters which I feel able to judge, the literature is well covered and accurately summarised.

Murton and Westwood state at the beginning that their aim is to bring together work on the proximate (immediate causal mechanisms) and ultimate (survival value) factors determining breeding seasons in birds. This is a worthwhile aim, although in practice the two questions are dealt with in separate chapters, and attempts to link the two often result in rather extensive speculation which leaves the reader feeling slightly uneasy. Chapter 14, on "Evolutionary Aspects of Photoperiodism", illustrates the point. The authors start with a straightforward and most interesting observation: the breeding seasons of different species of captive wildfowl held at Slimbridge correlate with their natural breeding latitudes. All the species experience the same photoperiod at Slimbridge, but those from higher latitudes breed later. The suggested conclusion is that there are built-in differences in response to photoperiod which are evolutionary adaptations to breeding at different latitudes. This much is fine, but the authors then go on to make elaborate speculations about "neural thresholds of a single hypothalamic oscillator", and about primitive species in which "there is some simple relationship between the phasing of gonadotrophin secretion and thyroid activity so that about a particular daylength threshold only gonadotrophin effects prevail."

The authors also use their observed correlation between latitude and breeding cycle to work backwards to

speculate on the evolutionary relationships and geographical origin of particular species. Here the arguments become tenuous. For example, on the one hand it is argued that photoperiodic responses are adaptive, and thus presumably flexible in evolutionary time, but on the other hand it is suggested geese have hardly ever succeeded in invading the tropics because they have an inappropriate photoperiodic response. This sort of pitfall in evolutionary arguments is not unique to the present book but Murton and Westwood seem to stumble into it with regularity.

The mixture of fact and speculation is not confined to sections of the book dealing with evolutionary questions. A typical example comes from the section on photorefractoriness on p320: "While it remains likely that LH and FSH secretion rhythms depend on the same oscillator mechanism it can be postulated that TSH (and ACTH) can be independently phased and so may assume varying phase relationships...". It is not always easy to tell where the facts end and the speculation starts, and this is one reason why I am not sure at whom the book is aimed. The preface optimistically suggests that senior undergraduates will use the book, but most of it is far too difficult or obscure for such students. The section introducing circadian rhythms soon gets into difficult sentences such as "The maximum permissible phase shift to affect entrainment is 180°, and there is a 180° forbidden zone when a driven oscillator would transfer energy to, instead of receive energy from, the driver (this is an approximation for sinusoidal oscillations and in special cases deviations occur)". On the other hand, the authors frequently fall back on phrases such as "the above results are rather difficult to understand without reference to detailed results". This may well frustrate the research worker hoping to get a really exhaustive treatment of his own area. My conclusion is that the book will be mainly used as a reference guide to the areas covered. Undergraduates will have to go to more straightforward accounts for the basic facts, and research workers will be in a position to judge the speculative sections and follow up in detail the issues which are not treated in enough depth. The book deserves to do well as a reference work, because in spite of my reservations about some aspects, it is a bold and impressive attempt to bring together widely differing fields of research.

John Krebs

John Krebs is at the Edward Grey Institute of Field Ornithology, University of Oxford, UK.

Recent scientific and technical books

Biology

- JAMESON, D. L. (edited by). *Genetics of Speciation*. (Benchmark Papers in Genetics/9.) Pp.xvii + 336. ISBN-0-87933-302-3. (Stroudsburg, Penn.: Dowden, Hutchinson and Ross, Inc., 1977. Distributed by Academic Press, New York and London.) \$25.
- KINGDON, Jonathan. *East African Mammals: An Atlas of Evolution in Africa*. Vol. 3. Part A: Carnivores. Pp.viii + 476. ISBN-0-12-408303-X. (London and New York: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) £38; \$74.25.
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- LISTER, R. E. *Diagnostic Testing in Advanced Biology*. Pp.168. ISBN-0-340-21227-6. (London: Hodder and Stoughton, 1978.) £2.25.
- MALLINSON, Jeremy. *The Shadow of Extinction: Europe's Threatened Wild Mammals*. Pp.xii + 224. ISBN-0-333-19214-1. (London and Basingstoke: Macmillan London, Ltd., 1978.) £5.50 net.
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- THELLIER, M., MONNIER, A., DEMARTY, M., and DAINY, J. *Echanges Ioniques Transmembranaires Chez les Vegetaux/Transmembrane Ionic Exchanges in Plants*. (Colloque International du Centre National de la Recherche Scientifique, Universités de Rouen et Paris VIII, 5-11 Juillet 1976, No. 258. Pp.607. ISBN-2-902618-44-1. (Paris: Editions du Centre National de la Recherche Scientifique, et Publications de l'Université de Rouen, 1977.) 180 francs.
- VOLKENSTEIN, M. V. *Molecular Biophysics*. Pp.ix + 621. ISBN-0-12-723150-1. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$36.50; £25.90.
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- WHITE, Michael J. D. *Modes of Speciation*. (A Series of Books in Biology.) Pp.455. ISBN-0-7167-0284-3. (San Francisco and Reading: W. H. Freeman and Company, 1978.) £18.50.
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- BEEMER, A. M., BEN-DAVID, A., KLINGBERG, M. A., and KUTTIN, E. S. (edited by). *Host-Parasite Relationships in Systemic Mycoses. Part 2: Specific Diseases and Therapy*. (Contributions to Microbiology and Immunology, Vol. 4.) (Proceedings of the 21st Annual OHOLO Biological Conference, Ma'alot, Israel, March 28-31, 1976.) Pp.xiv + 194. ISBN-3-8055-2444-7. (Basel, London and New York: S. Karger, 1977.) Sfr./DM 74; \$33.
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- GREEP, Roy O. (project director). *KOBLINSKY, Marjorie A. (assistant project director). Frontiers in Reproduction and Fertility Control: A Review of the Reproductive Sciences and Contraceptive Development*. Sponsored by the Ford Foundation. Pp.xiii + 580. ISBN-0-262-07068-5. (Cambridge, Mass. and London: The MIT Press, 1977.) £21.
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Psychology

FISCHER, Joel, and GÖCHROS, Harvey L. *Handbook of Behavior Therapy with Sexual Problems*. Vol. 1: General Procedures. Pp. liii + 1-259. ISBN-0-08-020373-6. Vol. 2: Approaches to Specific Problems. Pp. xvi + 261-604. ISBN-0-08-020374-4. (Pergamon General Psychology Series.) (Oxford and New York: Pergamon Press, 1977.) £11.25 each volume.

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Sociology

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History of science

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General

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Astronomy

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NEWTON, Jack. *Deep Sky Objects: a Photographic Guide for the Amateur*. Pp. 160. ISBN-0-88904-081-8. (Toronto: Gall Publications, 1293 Gerrard Street, East, 1977.) Soft cover \$3; Hard cover £5.

PROCEEDINGS OF THE EIGHTH LUNAR SCIENCE CONFERENCE, Houston, Texas, March 14-18, 1977. Compiled by The Lunar Science Institute, Houston, Texas. Vol. 1: The Moon and the Inner Solar System. Pp. 1-1304. 25 plates. xxiv. Vol. 2: Petrographic Studies of Mare and Highland Rocks. Pp. 1305-2654. xxiv. Vol. 3: Planetary and Lunar Surfaces. Pp. 2655-3965. xxvi. (*Geochimica et Cosmochimica Acta*, Supplement 8.) ISBN-0-08-022052-5. (Oxford and New York: Pergamon Press, 1977.) £100.

UNSOLD, Albrecht. *The New Cosmos*. Translated by R. C. Smith, based on the translation by W. H. McCrea of the 1st Edition. (Heidelberg Science Library.) Pp. xii + 451. ISBN-3-540-90223-6. (Berlin and New York: Springer-Verlag, 1977.) DM 37; \$18.50.

Physics

ANDRE, Jean-Marie, DELHALE, Joseph, and LAJIK, Janos (edited by). *Quantum Theory of Polymers*. (Proceedings of the NATO Advanced Study Institute on Electronic Structure and Properties of Polymers, held at Namur, Belgium, 31 August-14 September, 1977.) (NATO Advanced Study Institutes Series, Series C: Mathematical and Physical Sciences, Vol. 39.) Pp. xi + 376. ISBN-90-277-0870-3. (Dordrecht, Holland and Boston, Mass.: D. Reidel Publishing Company, 1978. Published in co-operation with NATO Scientific Affairs Division.) Dfl. 85; \$35.

BAISOV, N. G. (edited by). *Electrical and Optical Properties of III-V Semiconductors*. (Proceedings (Trudy) of the P.N. Lebedev Physics Institute, Vol. 89.) Pp. viii + 118. ISBN-0-306-10944-1. (New York and London: Consultants Bureau, 1978.) \$39.

BAISOV, N. G. (edited by). *Superconductivity*. (Proceedings (Trudy) of the P. N. Lebedev Physics Institute, Vol. 86.) Translated from Russian by G. D. Arkharov. Pp. ix + 168. ISBN-0-306-10939-5. (New York and London: Consultants Bureau, 1977.) \$35.

COOKS, R. G. *Collision Spectroscopy*. Pp. xiv + 458. ISBN-0-306-31044-9. (New York and London: Plenum Press, 1978.) \$54.60.

CROXTON, Clive A. (edited by). *Progress in Liquid Physics*. Pp. viii + 592. ISBN-0-471-99445-6. (Chichester and New York: John Wiley and Sons, 1978.) £25.

EHRENREICH, Henry, SEITZ, Frederick, and TURNBULL, David. *Solid State Physics: Advances in Research and Applications*. Vol. 32. Pp. xiv + 328. ISBN-0-12-607732-0. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$26; £16.90.

ELWELL, D., and POINTON, A. J. *Physics for Engineers and Scientists*. Second edition. Pp. xi + 356. ISBN-0-85312-093-5. (Chichester: Ellis Horwood, Ltd., 1978. Distributed by Halsted Press, a Division of John Wiley and Sons, New York and London.) £15.

FLEMING, Phyllis J. *Physics*. (Addison-Wesley Series in Physics.) Pp. xii + 557. ISBN-0-201-02472-1. (Reading, Mass. and London: Addison-Wesley Publishing Company, 1978.) £12.75.

HALL, S. J., and IRVINE, J. M. (edited by). *Nuclear Structure Physics*. (Proceedings of the Eighteenth Scottish Universities Summer School in Physics, St. Andrews, August 1977. A NATO Advanced Study Institute.) Pp. 724. ISBN-0-905945-01-8. (Edinburgh: SUSSP Publications, University of Edinburgh, Department of Physics, 1978.) £15.

HERGLOTZ, H. K., and BIRKS, L. S. *X-Ray Spectrometry*. (Practical Spectroscopy: a Series.) Pp. xi + 518. ISBN-0-8247-6625-3. (New York and Basel: Marcel Dekker, Inc., 1978.) \$49.50.

LIGHTHILL, James. *Waves in Fluids*. Pp. xv + 504. ISBN-0-521-21689-3. (Cambridge, London and New York: Cambridge University Press, 1978.) £17.50 net.